Volume LXXIV Number 3

THE BOTANICAL GAZETTE

Editor: JOHN M. COULTER

NOVEMBER 1922

Physiological Studies of Effects of Formaldehyde on Wheat — W. M. Atwood — 233
(With twelve figures)

Development of the Geoglossaceae — G. H. Duff — 264
(With Plates VIII–XII)

A Morphological Study of the Umbelliferae. Contributions from the Hull Botanical Laboratory 298 — Hilary Stanislaus Jurica — 292
(With Plates XIII–XIV)

Determination of Moisture Content of Expressed Plant Tissue Fluids — Ross Aiken Gortner and Walter F. Hoffman — 308

Moisture Content of Peach Buds in Relation to Temperature Evaluations — Earl S. Johnston — 314
(With two figures)

Alternaria from California — D. G. Milbraith — 320
(With two figures)

Basisporium Gallarum Moll., a Parasite of the Tomato — G. B. Ramsey — 325
(With eleven figures)

Briefer Articles

Sagenopteris, a Mesozoic Representative of the Hydropteraceae — Edward W. Berry — 329
(With one figure)

A Bisporangiate Sporophyll of Lycopodium lucidulum — A. W. Dupler — 331
(With one figure)

A Simple Apparatus for Controlling Temperatures — Ernest E. Hubert — 333
(With one figure)

Current Literature

Book Reviews — 335

Notes for Students — 336

The University of Chicago Press
CHICAGO, ILLINOIS, U.S.A.

THE CAMBRIDGE UNIVERSITY PRESS, *London*
THE MARUZEN-KABUSHIKI-KAISHA, *Tokyo, Osaka, Kyoto, Fukuoka, Sendai*
THE MISSION BOOK COMPANY, *Shanghai*

NEW YORK BOTANICAL GARDEN LIBRARY 1922

Volume LXXIV · Number 3

THE BOTANICAL GAZETTE

A MONTHLY JOURNAL EMBRACING ALL DEPARTMENTS OF BOTANICAL SCIENCE

EDITED BY

JOHN M. COULTER

With the assistance of other members of the botanical staff of the University of Chicago

Issued November 23, 1922

The Botanical Gazette is published in eight issues annually (omitting January and February, and July and August) by the University of Chicago at the University Press, 5750 Ellis Avenue, Chicago, Ill. ¶The subscription price is $9.00 per year; the price of single copies is $1.25. Orders for service of less than a half-year will be charged at the single-copy rate. ¶Postage is prepaid by the publishers on all orders from the United States, Mexico, Cuba, Porto Rico, Panama Canal Zone, Republic of Panama, Dominican Republic, El Salvador, Bolivia, Colombia, Honduras, Nicaragua, Peru, Hawaiian Islands, Philippine Islands, Guam, Samoan Islands, Shanghai. ¶Postage is charged extra as follows: For Canada, 30 cents on annual subscriptions (total $9.30); on single copies 4 cents (total $1.29); for all other countries in the Postal Union, 60 cents on annual subscriptions (total $9.60), on single copies 8 cents (total $1.33). ¶Patrons are requested to make all remittances payable to The University of Chicago Press in postal or express money orders or bank drafts.

The following are authorized to quote the prices indicated:

For the British Empire: The Cambridge University Press, Fetter Lane, London, E.C. 4, England. Yearly subscriptions, including postage, £2 13s. each; single copies, including postage, 7s. 4d. each.

For Japan and Korea: The Maruzen-Kabushiki-Kaisha, 11 to 16 Nihonbashi Tori Sanchome, Tokyo, Japan. Yearly subscriptions, including postage, Yen 20.16 each; single copies, including postage, Yen 2.80 each.

For China: The Mission Book Company, 13 North Szechuen Road, Shanghai. Yearly subscriptions, $9.00; single copies, $1.25, or their equivalents in Chinese money. Postage extra, if mailed direct outside of Shanghai, on yearly subscriptions 60 cents, on single copies 8 cents.

Claims for missing numbers should be made within the month following the regular month of publication. The publishers expect to supply missing numbers free only when losses have been sustained in transit and when the reserve stock will permit.

Business correspondence should be addressed to The University of Chicago Press, Chicago, Ill.

Communications for the editor and manuscripts should be addressed to the Editor of the BOTANICAL GAZETTE, The University of Chicago, Chicago, Ill.

Contributors are requested to write scientific and proper names with particular care, to use the metric system of weights and measures, and in citations to follow the form shown in the pages of the BOTANICAL GAZETTE.

Papers in excess of thirty-two printed pages are not accepted unless the author is willing to pay the cost of the additional pages, in which case the number of the pages in the volume is correspondingly increased. Carbon copies of manuscripts will not be accepted.

Illustrations are furnished without cost to author only when suitable originals are supplied. Plates in excess of four are not accepted unless the author is willing to bear the additional cost. A copy of the suggestions made in the April number 1916, will be sent on application. It is advisable to confer with the editors as to illustrations required in any article to be offered.

Separates if desired, must be ordered in advance of publication. A table showing approximate cost of separates is printed on an order blank which accompanies the proof; a copy will be sent on request.

Entered as second-class matter August 21, 1896, at the Post-Office at Chicago, Illinois, under the Act of March 3, 1879.

Acceptance for mailing at special rate of postage provided for in Section 1103, Act of October 3, 1917, authorized on July 15, 1918.

PRINTED IN THE U.S.A.

The University of Chicago

Nature-Study Series

By ELLIOT R. DOWNING

Associate Professor of Natural Science in the School of Education of the University of Chicago

Already Published

A Source Book of Biological Nature-Study

Cloth, $3.00; postpaid $3.20

The material is organized with special reference to the training of the teacher and the presentation of the subject to the pupil. In all the varied uses to which it may be put the 338 sketches and photographs add greatly to its effectiveness and charm.

"One fact strikes us with overwhelming force—namely, how immense an array of valuable and instructive matter can be presented to the student without a trace of dullness or pedantry . . . . the volume is literally a moving picture of natural history and a wide open door to vaster and more comprehensive fields of research."—*Chicago Daily News* for June 4, 1919.

A Field and Laboratory Guide in Biological Nature-Study

Paper covers, loose leaf, $1.50; postpaid $1.60

Intended for the use of students who are preparing to teach, and of teachers themselves. Deals with those groups of plants and animals that are of most interest to pupils.

"It gives specific suggestions and directions for field work. There are three keys: to autumn weeds, to trees, and to birds. There is much data as to methods given throughout the book, the outcome of years of work, which, brought together as it is under one cover, is invaluable to the inexperienced teacher."—*School Science and Mathematics* for May, 1919.

A Field and Laboratory Guide in Physical Nature-Study

Paper cover, loose leaf, $1.50; postpaid $1.60

Designed to acquaint the student with the rich field of physical science so often neglected in nature-study because of overemphasis on living things. The material here presented has been used by the author for many years in his teacher-training classes.

"A wise teacher will find many ways in which to use this manual, and many a boy or girl will find its pages suggestive and delightful."—*Education*, May, 1920.

"It should be in the hands of every teacher in the land who has to do with seventh and eighth grades or with high-school physics."—*Nature-Study Review* for April, 1920.

A Naturalist in the Great Lakes Region

Educational Edition, cloth, $2.50, postpaid $2.60
Flexible binding, pocket size, $3.50; postpaid $3.70

This book gives an interesting description of the flora and fauna of the Great Lakes region and traces the development of the geological and physiographic factors which have affected the distribution of the plants and animals.

Other Volumes Are in Preparation

Address Correspondence to

THE UNIVERSITY OF CHICAGO PRESS
CHICAGO - - - - - - - - ILLINOIS

Cambridge University Press

Lichens. By ANNIE LORRAIN SMITH, F.L.S., Acting Assistant, Botanical Department, British Museum. With 135 illustrations. Royal 8vo. Price on application. Cambridge Botanical Handbooks.

Fungi. Ascomycetes, Ustilaginales, Uredinales. By HELEN GWYNNE-VAUGHAN, D.B.E., LL.D., D.Sc., F.L.S. With a frontispiece and 196 text-figures. Royal 8vo. Price on application. Cambridge Botanical Handbooks.

The Classification of Flowering Plants. By ALFRED BARTON RENDLE, M.A., D.Sc., F.L.S., Keeper of the Department of Botany, British Museum. Vol. I. Gymnosperms and Monocotyledons. Demy 8vo. With 187 illustrations. $5.00. Cambridge Biological Series.

A Manual and Dictionary of the Flowering Plants and Ferns. By J. C. WILLIS, M.A., Sc.D. Fourth edition. Crown 8vo. $8.40. Cambridge Biological Series.

The Elements of Botany. By SIR FRANCIS DARWIN, Sc.D., M.B., F.R.S., Fellow Christ's College. Second edition. With 94 illustrations. Crown 8vo. $2.60. Cambridge Biological Series.

Practical Physiology of Plants. By SIR FRANCIS DARWIN, Sc.D., F.R.S., and E. HAMILTON ACTON, M.A. Third edition. With 45 illustrations. Crown 8vo. $2.10. Cambridge Biological Series.

Botany. A textbook for Senior students. By D. THODAY, M.A., Lecturer in Physiological Botany and Assistant Director of the Botanical Laboratories in the University of Manchester. Revised and enlarged edition. With 230 text-figures. Large crown 8vo. $2.60.

The Ferns of South Africa containing descriptions and figures of the ferns and fern allies of South Africa. By THOMAS R. SIM. Second edition. With 186 plates. Demy 8vo. $10.00.

Mendel's Principles of Heredity. By W. BATESON, M.A., F.R.S., V.M.H., Director of the John Innes Horticultural Institution. Third impression with additions. With 3 portraits, 6 colored plates, and 38 figures. Royal 8vo. $5.00.

Water Plants. A Study of Aquatic Angiosperms. By AGNES ARBER, D.Sc., F.L.S. Royal 8vo. With a frontispiece and 171 text-figures. $10.00.

Devonian Floras. A Study of the Origin of Cormophyta. By E. A. NEWELL ARBER, D.Sc., F.L.S. With a preface by D. H. SCOTT, F.R.S. Demy 8vo. With a frontispiece and 47 text-figures. $7.00.

Practical Plant Biochemistry. By MURIEL WHELDALE ONSLOW. Royal 8vo. $6.40.

Published by the Cambridge University Press (England)
The Macmillan Co., Agents in the United States
64 and 66 Fifth Avenue, New York City

New and Revised Edition

Methods in Plant Histology

By CHARLES JOSEPH CHAMBERLAIN, PH.D.
Professor of Botany in the University of Chicago

This book (third edition, completely revised) contains complete directions for collecting and preparing plant material for microscopic investigation. Killing and fixing, sectioning, staining; the paraffin, Venetian turpentine, celloidin, and glycerine methods are treated in detail, and chapters on making photomicrographs and lantern slides have been added. Ten years' experience with the Venetian turpentine method has brought it to a high degree of efficiency; the paraffin method has been greatly improved; the notes on staining have kept pace with cytological research, and the directions for finding material are more extensive.

The book may be used in classes under an instructor, but the directions are so explicit that students working alone should soon learn to make first-class preparations.

With the exception of the sections on the glycerine and celloidin methods, this third edition is practically a new book.

Illustrated, cloth; $3.25, postpaid $3.37

The University of Chicago Press
Chicago - - - - Illinois

Authority in floristics offers supply of all kinds botanical material. Scientific execution guaranteed.

Write and send orders to

Dr. Hans Steffen, Allenstein (Ostpreussen) Germany
References: The University of Koenigsberg

If you do not receive our advertising matter regularly, ask to be put on our mailing list.

THE UNIVERSITY OF CHICAGO PRESS

From the Cambridge University Press

THE GENUS IRIS

By WILLIAM RICKATSON DYKES

With 48 colored plates and 30 line drawings in the text

This magnificent work brings together the available information on all known species of Iris. The most striking feature of the book is the life-size colored plates, reproduced from originals drawn from living plants—making it a volume of remarkable beauty as well as of great scientific importance.

254 pages, demi folio, half morocco; price on request.

The University of Chicago Press
Chicago - - - - - Illinois

PLANT GENETICS

By **JOHN M. COULTER**
Head of the Department of Botany in the University of Chicago

and **MERLE C. COULTER**
Assistant Professor of Plant Genetics in the University of Chicago

¶ This book has been written to meet an increasing need among botanical students. Such students in these days, in whatever phase of botany they may be specializing, find it necessary to read with understanding much of the literature of plant genetics, because it is becoming increasingly significant in all botanical problems. This means that teachers and investigators must be able to command the literature of plant genetics, much of which has been so complex as to be a closed book for the uninitiated. *Plant Genetics* is an attempt to open this subject to botanical students.

¶ The book is not intended to be a thorough, authoritative text, but a relatively simple presentation of the more significant investigations on plant genetics which will initiate the student into the subject. Material dealing with some highly specialized phases of genetics and material that is very complex has been purposely omitted for pedagogical reasons. In short, the book is an easy introduction to plant genetics.

Cloth; $1.50, postpaid $1.60

THE UNIVERSITY OF CHICAGO PRESS
CHICAGO - - - - - - - ILLINOIS

THE LIVING CYCADS

By Charles Joseph Chamberlain

Professor of Botany in the University of Chicago

This study includes investigations extending over fifteen years, that necessitated trips to Cuba, New Mexico, Australia, and Africa. The author studied all the genera and many of the species in the field, and preserved much material for later study in the laboratory.

In the first part of the book is given an account of the distribution, general appearance, and field conditions of the cycads, together with some of the experiences of an investigation involving much travel in distant and varied countries. In the second part is presented the life-history of the group based largely on the author's own observations in the field and laboratory. Part III is devoted to the evolution and phylogeny of the cycads, the opportunity for such a study being exceptionally favorable because the ancestry can be traced back through geological periods, and because the extinct predecessors of the cycads are the best known of the fossil plants.

Cloth; $1.50, postpaid $1.60

THE UNIVERSITY OF CHICAGO PRESS
CHICAGO - - - - - - - ILLINOIS

Second Impression

The Anatomy of Woody Plants

(With 306 figures and illustrations)

***By* E. C. JEFFREY**

Professor of Plant Morphology in Harvard University

The author is easily the leading authority on the anatomy of plants. His book aims to describe, analyze, and discuss the anatomical structure of the most important families of plants. It does for the anatomy of plants what so many textbooks have done for the human anatomy. The book will be of great service, not only to botanical instruction everywhere, but also to paleobotany, for the study of vascular anatomy has revolutionized that subject.

Cloth, $4.75, postpaid $4.87

THE UNIVERSITY OF CHICAGO PRESS
CHICAGO - - - - - - - - - - ILLINOIS

VOLUME LXXIV NUMBER 3

THE
BOTANICAL GAZETTE

November 1922

PHYSIOLOGICAL STUDIES OF EFFECTS OF FORMALDEHYDE ON WHEAT[1]

W. M. ATWOOD

(WITH TWELVE FIGURES)

Introduction

Copper sulphate and formaldehyde have been most commonly used as fungicides in the treatment of seed wheat. The choice between the two has often been determined by local custom and prejudice, while in other cases climatic differences have been thought to be worthy of consideration in the selection. During the twenty years, approximately, that formaldehyde has been used as a dip or spray, as a gas (**44, 53**) or with steam (**39**), reports have differed radically in the degree of favor with which it has been viewed.

One group of experimenters has reported injury to germination or seedling vitality, or both, following the use of formaldehyde. STEPHENS of the Sherman County Branch Experiment Station at Moro, Oregon, has consistently reported injury in his station reports since 1913 (**50**). In 1917 he noted that 18.5 per cent of the seed wheat was killed. He has made the further important observation that in many cases seedlings may progress in development but with lessened vegetative vigor. HEALD and WOOLMAN (**31**) found germination reductions at concentrations of 30–40 gallons of water

[1] The project on which the present paper is based was financed under the Adams Fund, to which acknowledgment is hereby made.

to the pint of formaldehyde. In Utah, STEWART and STEPHENS (52) noted vitality reductions in wheat, barley, and oats, but thought the advantages outweighed the injury. MACKIE (37) of California noted that seed stored after treatment uniformly showed poor germination. Even with proper drying the tissues appeared hardened, causing retardation and distortion of the young seedlings. Varying degrees of injury have been reported by many different investigators (14, 19, 20, 21, 22, 53, 57, 58).

On the other hand, formaldehyde has been approved in varying measures by different investigators, some of whom recognized the dangers and injury in some cases, but have felt that the advantages outweighed the injury. The War Emergency Board of American plant pathologists found little injury from formaldehyde except when the concentrations were higher than the usual 1 part to 320 parts of water, or when the other common precautions in treatment had not been observed. This work was based on the reports of seventeen experiment stations, and is probably the most complete and uniformly secured set of data available from so large an area of country (35). Many other workers in America and Europe have reported in similar vein (6, 16, 32, 36, 38, 40, 45, 51, 55).

Within the past two years two most interesting papers have appeared, in which the possibilities of avoiding injury from formaldehyde treatment have been suggested. BRAUN (13) finds the injury apparently much diminished by not treating the grain until imbibed with water. It is believed that exterior disinfection is thus attained, and a much less amount of formaldehyde enters the grain under these conditions. Miss HURD (33) believes that when seeds are treated in formaldehyde and subsequently allowed to dry, the polymer paraformaldehyde is deposited on the seed coat with serious eventual injury. Here, instead of the "pre-soak," we have the recommendation of washing in water subsequent to treatment to avoid the harmful paraformaldehyde deposits. With the work of Miss HURD there appears to be a better explanation than formerly of the source of the injury of formaldehyde to seeds. Amid a wide diversity of opinions as to the value of the disinfectant, and with differing recommendations for reduction in treatment injury, it seemed altogether desirable that something be learned as

to the exact nature of the effect exerted by formaldehyde on the physiological processes of seeds, as shown by wheat. Accordingly, the Oregon Experiment Station has been occupied in such studies, during parts of the past three seasons, and inasmuch as local conditions have necessitated the temporary discontinuance of this work, it was thought well to report the results already obtained.

Experimentation

Wheat for these studies was kindly furnished by Mr. STEPHENS of the Oregon Branch Experiment Station. In order that the behavior of this wheat in relation to formaldehyde might be known, it was thought advisable first to determine the effect on germination of varying the concentration and also the time of treatment. The formaldehyde used was the ordinary commercial material, the strength of which was determined according to the method outlined by HAYWOOD and SMITH (30), and found to contain 39.3 parts per hundred by volume of the formaldehyde gas.

In the studies of the effect of varying the time of treatment, the period was varied from 5 to 300 minutes of soaking in formaldehyde 1–320. The number of seeds used was 10,800, one-third being grown in blotters in the customary manner, one-third in soil in porous clay germinators indoors, and one-third outdoors in pots of soil exposed to the weather and a temperature between 40° and 60° F. The indoor samples were grown in the laboratory, and, as might be expected, germination was much more prompt at the higher temperature. It was found that the time of dip between twenty and forty minutes only slightly reduced the germination percentages. A somewhat greater drop in the curves (figs. 1, 2) occurs as the time is lengthened up to four hours, although the drop is not great in most cases. The seeds germinated in soil displayed a somewhat greater percentage of injury, as measured by appearance above soil, than was true of the samples grown in blotters. This difference between the behavior of formaldehyde treated seeds when germinated in soil and in blotters was noted by CRANEFIELD (20) in studying the effect of the fungicide on oats. He found the injury in oats grown in the soil averaged four times greater than that of seed grown in blotters. The explanation of this difference in

apparent injury was given by WALLDEN (56), who thought injuries to the coleoptile, making it difficult to pierce the soil, do not prevent the germination of seeds in blotters. Miss HURD (33), after making a similar observation, expresses preference for the blotter studies, which she believes show more clearly the distortion incident to

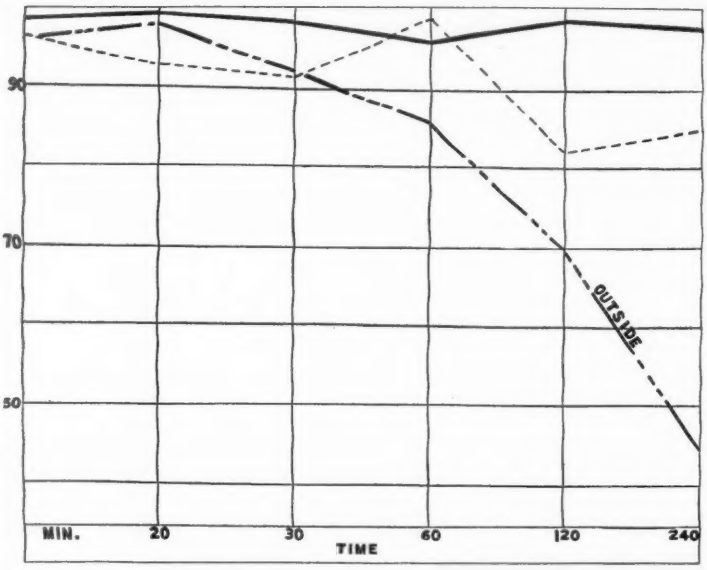


FIG. 1.—Effect of time of soaking wheat on percentage germination, Hybrid 128 wheat from Moro, Oregon: solid line shows percentage germination in blotters, broken lines in soil indoors and outside; formaldehyde mixed 1–320 parts of water; summary of 3600 seeds tested.

injury, even though the percentage stand which would be attained under field conditions by this method could only be estimated.

In varying the concentrations of formaldehyde, treatment was for ten minutes at 20° C., and the concentrations were varied from 40 to 320 parts of water to 1 part of formaldehyde. Uniform dropping in germination occurred in all cases with increasing concentrations. As compared with the water dipped controls, there was little injury apparent at the usual concentration of 1–320; but with a concentration of 1–160 the germination curves began to

fall, and at 1–40 the germination was cut from 40–60 per cent, both in the blotters and in the soil. Here again, as in the previous series, the injury was greatest in the outdoor soil, less in soil indoors, and least of all in the blotter tests (figs. 3, 4).

Formaldehyde readily forms various polymers (**8, 25**). On standing in the cool a flocculent white precipitate forms readily,

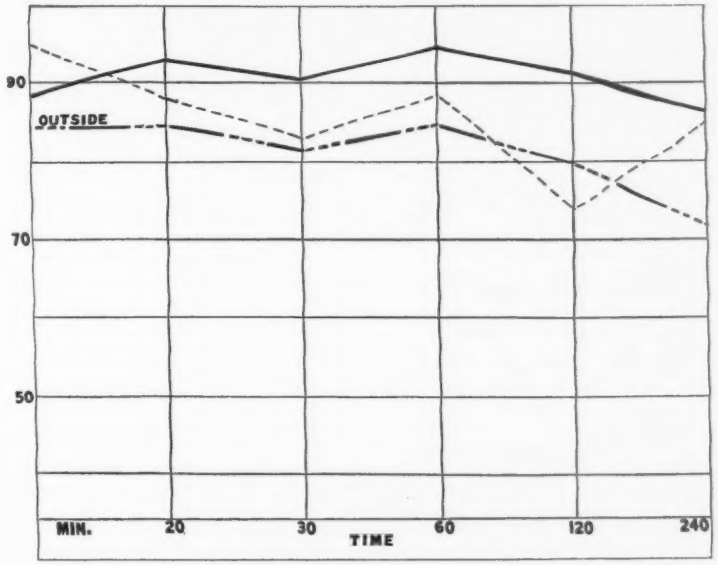


FIG. 2.—Effect of time of soaking wheat on percentage germination, Turkey Red wheat from Moro, Oregon: solid line shows percentage germination in blotters, broken lines in soil indoors and outside; formaldehyde mixed 1–320 parts of water; summary of 3600 seeds tested.

or on concentration of the commercial solutions. This is ordinarily referred to as paraformaldehyde, although the various polymers are probably often found more or less associated, and means for the identification of the various forms are not well known. Efforts have been expended toward developing methods to prevent such polymerization (**28**), but these methods have not been adopted in general. If wheat is dusted with the white flakes of this so-called paraformaldehyde, serious injury results. Turkey Red so treated

gave in one series of tests 9.5 per cent germination in blotters and 15 per cent in soil, as compared with 93.5 and 93 per cent respectively for the controls. This effect of the white polymer on the grain was noted by COONS and MCKINNEY (19), who found that it does not readily air out of grain but persists on it, so that its pres-

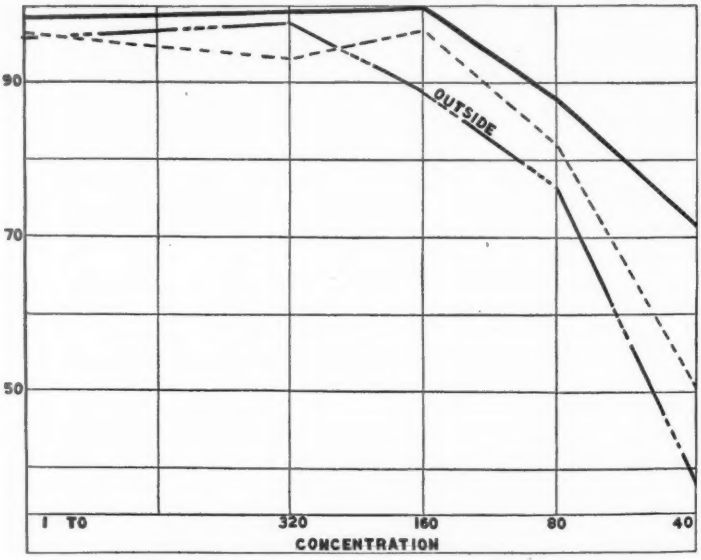


FIG. 3.—Effect of varied concentrations of formaldehyde on percentage germination, Hybrid 128 wheat from Moro, Oregon: solid line shows percentage for seeds germinated in blotters, broken lines for seeds in soil outside and indoors; ten minutes of soaking; summary of 3000 seeds tested.

ence could be demonstrated by an indicator after the grain had been exposed to the air of the laboratory for many months. Miss HURD (33) later has emphasized the extreme importance of the polymer as the possible channel through which injury from formaldehyde ordinarily results.

PERMEABILITY

It was recognized that it must be determined whether formaldehyde actually penetrates the coat of wheat. It has long been known that the seed coats of many seeds exhibit varying powers of exclu-

sion. BROWN (15) showed this to be conspicuously the case for barley, while SHULL (49) found semipermeability of seed coats a rather general situation. SCHROEDER (47) showed that the coat of wheat is permeable to the entry of mercuric chloride, iodine, alcohol, ether, chloroform, and acetic acid when in water solutions. Injury to the seed coat destroys this seed coat power of exclusion.

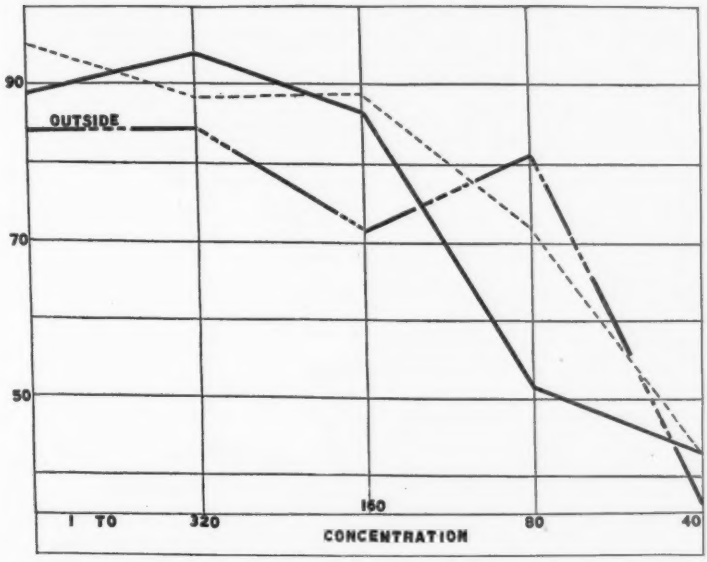


FIG. 4.—Effect of varied concentrations of formaldehyde on percentage germination, Turkey Red wheat from Moro, Oregon: solid line shows percentage for seeds germinated in blotters, broken lines for seeds in soil indoors and outside; ten minutes of soaking; summary of 3000 seeds tested.

Miss HURD (34) found that injuries from the entry of fungicides are much worse when seed coat cracks exist over the embryo.

Two methods were employed in studying the relation of the seed coat of wheat to formaldehyde entry. After various difficulties in technique, at the suggestion of Dr. E. M. HARVEY, the method was finally adopted of sealing the seeds one at a time to the end of small glass tubing, into which formaldehyde solution was placed. After allowing the seed to be in contact with the solution

for 3–4 days, the dry tip of the grain exterior to the tube was sectioned and treated directly with the Schryver formaldehyde reagent (29). With long periods of exposure to high concentrations of formaldehyde (1–8) penetration appears to be possible at either tip of the grain or on either face. The second method employed was to measure the degree of semipermeability of the seed coat indirectly by determining the weight increase of the seeds when soaked in distilled water and in formaldehyde respectively. For-

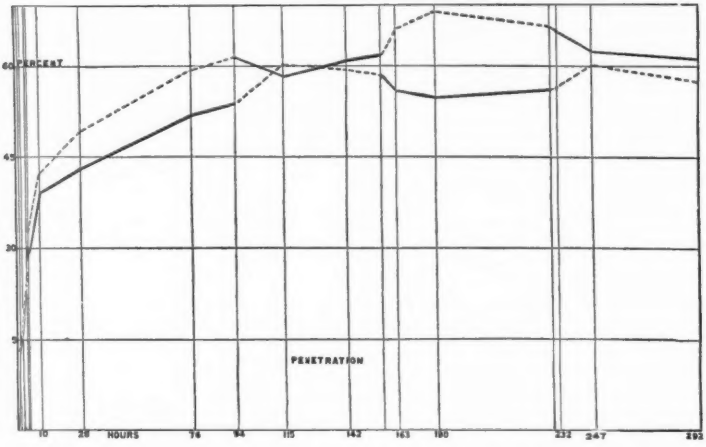


FIG. 5.—Comparative absorption by wheat of water and formaldehyde mixed 1 to 8 parts of water, Hybrid 128 wheat from Moro, Oregon: solid lines indicate percentages of weight increases when seeds were soaked in formaldehyde, broken lines when soaked in water.

maldehyde of high concentration (1–8) was used, in order to make more conspicuous any semipermeability differences of the coat toward the water and formaldehyde. In harmony with the results of BROWN (15), if seeds with semipermeable coats be placed in salt solutions, water will be taken up by imbibition until the inward force is offset by the equal outward osmotic force incident to the solution outside. If the two forces just balance each other, and if the coats be perfectly semipermeable, further soaking of the seeds in solution will not cause a rise in the curve indicating percentage weight increase, and the curve will continue horizontal. On the

other hand, if there is a gradual entry of the salt into the seed, the curve will flatten out quickly, and after changing the seeds back into pure water, the sudden and extensive rise of the curve as the water enters will be followed by a subsequent sinking as the salt gradually diffuses out of the seed again. In this way, by long and careful soaking of seeds in water and solutions of higher osmotic concentration, it is possible to determine whether the substance in solution is entering the seed coat. Frequent weighings are essential, each seed lot being dried of surface moisture with filter paper at each weighing. After the curves approach equilibrium, the seeds in either solution are transferred to the other one, with special attention to the behavior of the curves at this reversal of solutions. The weighings were made in triplicate with 3.5 gm. samples. Seventeen intervals, covering 292 hours of soaking, were followed by computation of percentages. A study of the resultant curves (fig. 5) leads to the conclusion that formaldehyde slowly penetrates the seed coat, and that when the grain is again transferred to distilled water, the formaldehyde gradually diffuses outward. These results are shown in table I. The variations at the time of reversal are by no means so great, even with this high concentration of formaldehyde,

TABLE I

DATA ON PENETRATION AS SUMMARIZED IN FIG. 5; PERCENTAGE WEIGHT INCREMENTS AT VARYING PERIODS OF SOAKING OF WHEAT

No.	Air dry weight	Hours of soaking																		
		1	2	4	4.75	10	28	76	94	115	142	157	163	180	229	232	247	292
1	3.5048	**11.9**	**15.7**	**22.9**	28.9	**38.5**	**43.4**	**53.2**	**55.1**	61.4	60.0	60.1	**57.3**	**55.4**	**57.1**	57.8	61.0	58.0
2	3.478	**11.9**	**15.6**	**23.6**	29.2	**39.2**	**43.4**	**51.6**	**53.3**	60.8	60.0	58.9	**56.4**	**54.7**	**56.5**	56.5	60.6	58.2
3	3.4106	**12.3**	**15.9**	**24.2**	30.1	**40.7**	**44.1**	**52.3**	**54.5**	60.0	59.5	58.6	**55.5**	**55.1**	**55.7**	57.1	59.8	57.2
4	3.2846	13.0	17.5	28.0	**32.8**	42.5	50.1	60.8	62.6	**59.5**	**62.1**	**63.1**	68.3	70.5	68.6	**66.4**	**63.2**	**61.4**
5	3.5406	13.3	18.1	27.9	**32.7**	42.9	49.6	59.4	61.6	**58.8**	**60.4**	**61.5**	65.9	68.9	64.4	**66.2**	**63.3**	**61.8**
6	3.487	13.2	17.5	27.5	**32.4**	41.9	49.1	58.9	61.1	**57.6**	**60.7**	**61.7**	65.7	68.2	67.8	**65.4**	**62.1**	**60.8**

* Figures printed in heavy type indicate soaking in formaldehyde; others in water.

for 3–4 days, the dry tip of the grain exterior to the tube was sectioned and treated directly with the Schryver formaldehyde reagent (29). With long periods of exposure to high concentrations of formaldehyde (1–8) penetration appears to be possible at either tip of the grain or on either face. The second method employed was to measure the degree of semipermeability of the seed coat indirectly by determining the weight increase of the seeds when soaked in distilled water and in formaldehyde respectively. For-

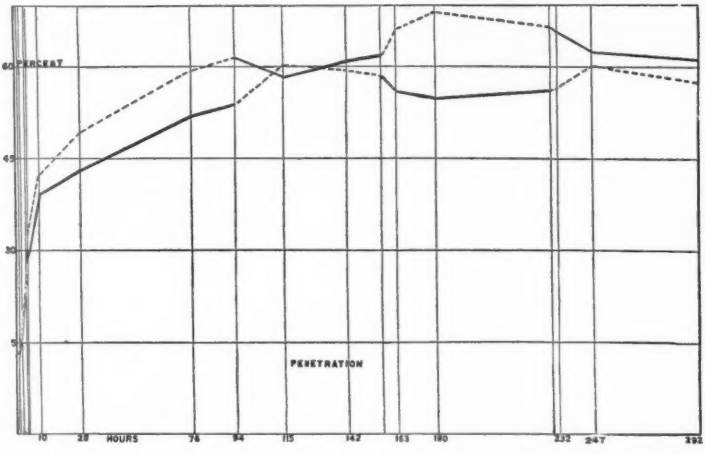


FIG. 5.—Comparative absorption by wheat of water and formaldehyde mixed 1 to 8 parts of water, Hybrid 128 wheat from Moro, Oregon: solid lines indicate percentages of weight increases when seeds were soaked in formaldehyde, broken lines when soaked in water.

maldehyde of high concentration (1–8) was used, in order to make more conspicuous any semipermeability differences of the coat toward the water and formaldehyde. In harmony with the results of BROWN (15), if seeds with semipermeable coats be placed in salt solutions, water will be taken up by imbibition until the inward force is offset by the equal outward osmotic force incident to the solution outside. If the two forces just balance each other, and if the coats be perfectly semipermeable, further soaking of the seeds in solution will not cause a rise in the curve indicating percentage weight increase, and the curve will continue horizontal. On the

other hand, if there is a gradual entry of the salt into the seed, the curve will flatten out quickly, and after changing the seeds back into pure water, the sudden and extensive rise of the curve as the water enters will be followed by a subsequent sinking as the salt gradually diffuses out of the seed again. In this way, by long and careful soaking of seeds in water and solutions of higher osmotic concentration, it is possible to determine whether the substance in solution is entering the seed coat. Frequent weighings are essential, each seed lot being dried of surface moisture with filter paper at each weighing. After the curves approach equilibrium, the seeds in either solution are transferred to the other one, with special attention to the behavior of the curves at this reversal of solutions. The weighings were made in triplicate with 3.5 gm. samples. Seventeen intervals, covering 292 hours of soaking, were followed by computation of percentages. A study of the resultant curves (fig. 5) leads to the conclusion that formaldehyde slowly penetrates the seed coat, and that when the grain is again transferred to distilled water, the formaldehyde gradually diffuses outward. These results are shown in table I. The variations at the time of reversal are by no means so great, even with this high concentration of formaldehyde,

TABLE I

DATA ON PENETRATION AS SUMMARIZED IN FIG. 5; PERCENTAGE WEIGHT INCREMENTS AT VARYING PERIODS OF SOAKING OF WHEAT

No.	Air dry weight	Hours of soaking																	
		1	2	4	4.75	10	28	76	94	115	142	157	163	180	229	232	247	292
1	3.5048	**11.9**	**15.7**	**22.9**	28.9	**38.5**	**43.4**	**53.2**	**55.1**	61.4	60.0	60.1	**57.3**	**55.4**	**57.1**	57.8	61.0	58.0
2	3.478	**11.9**	**15.6**	**23.6**	29.2	**39.2**	**43.4**	**51.6**	**53.3**	60.8	60.0	58.9	**56.4**	**54.7**	**56.5**	56.5	60.6	58.2
3	3.4106	**12.3**	**15.9**	**24.2**	30.1	**40.7**	**44.1**	**52.3**	**54.5**	60.0	59.5	58.6	**55.5**	**55.1**	**55.7**	57.1	59.8	57.2
4	3.2846	13.0	17.5	28.0	**32.8**	42.5	50.1	60.8	62.6	**59.5**	**62.1**	**63.1**	68.3	70.5	68.6	**66.4**	**63.2**	**61.4**
5	3.5406	13.3	18.1	27.9	**32.7**	42.9	49.6	59.4	61.6	**58.8**	**60.4**	**61.5**	65.9	68.9	64.4	**66.2**	**63.3**	**61.8**
6	3.487	13.2	17.5	27.5	**32.4**	41.9	49.1	58.9	61.1	**57.6**	**60.7**	**61.7**	65.7	68.2	67.8	**65.4**	**62.1**	**60.8**

* Figures printed in heavy type indicate soaking in formaldehyde; others in water.

as the writer (7) in earlier work has found to maintain with other grains in the presence of gram molecular solutions of sodium chloride. From the consistent behavior of these curves we must conclude that formaldehyde penetrates the coat of wheat, although such entry is slow. The work of BAKKE and PLAGGE (10) offers interesting confirmation of these conclusions. In their work the rate of entry of water and of 1–320 formaldehyde was compared. After a dip of fifteen minutes they found the weight increase about the same for the two, and concluded that water entry from 1–320 is not greatly different from the absorption of distilled water. The question of the comparative entry of water and of formaldehyde solutions becomes specially interesting in the light of COLLINS' (18) work with barley, which indicated that the entry of solutions, and hence the seat of selectivity, is in the germ end of the grain.

DIASTASE

In order to determine the effect on starch digestion of the presence of formaldehyde, a series of twenty-six test-tubes was filled each with 10 cc. of 25 per cent soluble potato starch solution. To all but two of the tubes 1 cc. of a filtered solution of Merck's medicinal diastase of 0.5 per cent concentration was added. To the test-tubes was then added 4 cc. of a formaldehyde solution varying in concentration from 1–1000 through 1–400, 1–320, 1–240, 1–160, 1–80, 1–40, 1–20, 1–10, 1–1, and pure 40 per cent commercial formaldehyde solution. Each condition was run in duplicate. These test-tubes were then incubated for 1.25 hours at 40° C. It was presumed at the beginning of these tests that it would be essential to determine the percentage of reducing sugars as a measure of the degree of digestion. It was found, however, that by modifying the methods used by APPLEMAN (3) and SHERMAN (48), it was easily possible to detect comparative differences in the amount of digestion by the deepness of coloration of the solution upon the addition of iodine. The stock solution of iodine as used eventually by dilution 10 cc. to 100 cc. of water was prepared with 1 gm. iodine, 5 gm. potassium iodide, and 50 cc. water. In the series enumerated the gradation of color was so obvious, from the deep blue of the check to the clear solutions where digestion was complete, that the

experiment was tried of giving to each tube at the end of the test a number value. No digestion (as in the tubes lacking diastase) was indicated by 10, complete digestion (no starch) by 0, and intermediate shades proportionately in between. It is not claimed that this method equals the accuracy of colorimetric technique, yet the differences were so pronounced that on checking over results with

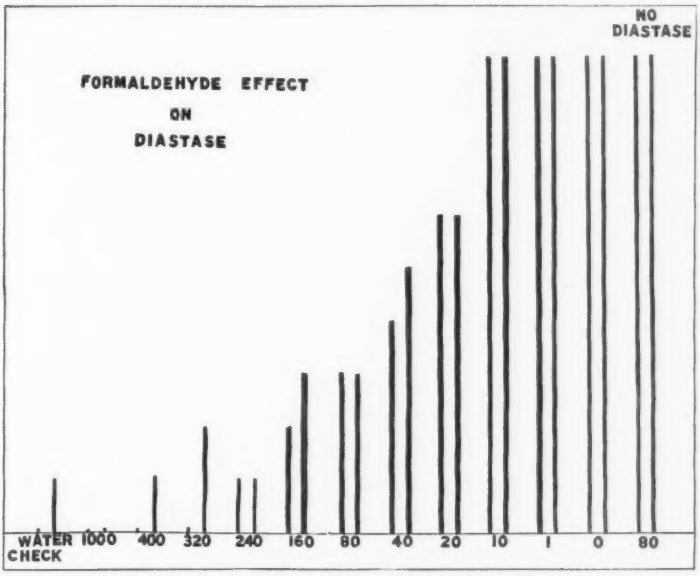


FIG. 6.—Effect of varying concentrations of formaldehyde on starch digestion: height of lines indicates amount of starch remaining undigested (all conditions shown in duplicate); 1 cc. 0.5 per cent solution Merck's diastase, 10 cc. 25 per cent soluble starch solution, and 4 cc. of varying concentrations of formaldehyde used in all but controls.

other observers who were unfamiliar with the conditions presented, it was thought that the situation did not justify the other method. Referring to fig. 6, in which these values are presented graphically, it will be observed that with the higher concentrations of formaldehyde the digestion is not greater in amount than that occurring in the check containing no diastase. Commencing with the concentration of 1–20 of formaldehyde, and running from that point down to

1–400, there is increasingly greater digestion found (less starch remaining). The question immediately arose as to whether the result was inhibition or a retardation of the rate of digestion. This was answered by running two series of digestions over a period of four hours at the same temperature as in the previous tests. To

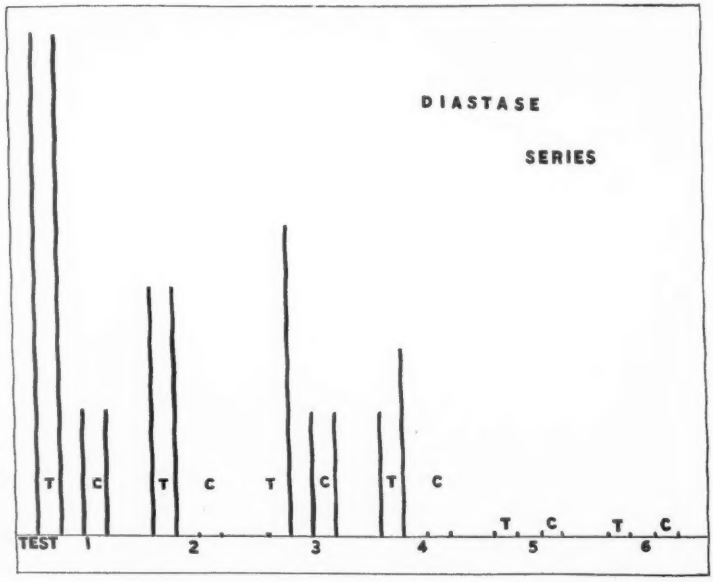


FIG. 7.—Time factor as related to starch digestion in presence of formaldehyde mixed 1–40 parts water: height of line indicates amount of starch remaining undigested (all conditions shown in duplicate); 1 cc. 0.5 per cent solution Merck's diastase, 10 cc. 25 per cent soluble starch solution, and 4 cc. formaldehyde used in tests (*T*), and a like amount water in controls (*C*); time periods in hours tests 1 to 6 respectively, 1.25, 1.5, 1.75, 2, 3, and 4 hours.

all the test-tubes of one series was added 1 cc. of the diastase solution, to one-half of the tubes was added 4 cc. of formaldehyde 1–40, and to the other half (control) an equal amount of water. Ten cc. of the 25 per cent soluble starch solution was placed in all tubes. The second series was prepared in the same manner, except that formaldehyde of 1–320 concentration was used. Four test-tubes from each series were removed every fifteen minutes, two contain-

ing formaldehyde and two controls, and the iodine test applied and results evaluated. Figs. 7 and 8 show that although digestion is markedly checked by 1–40 formaldehyde at the end of the first one and a quarter hours, digestion proceeds with further intervals of time, so that by three hours the digestion which had been com-

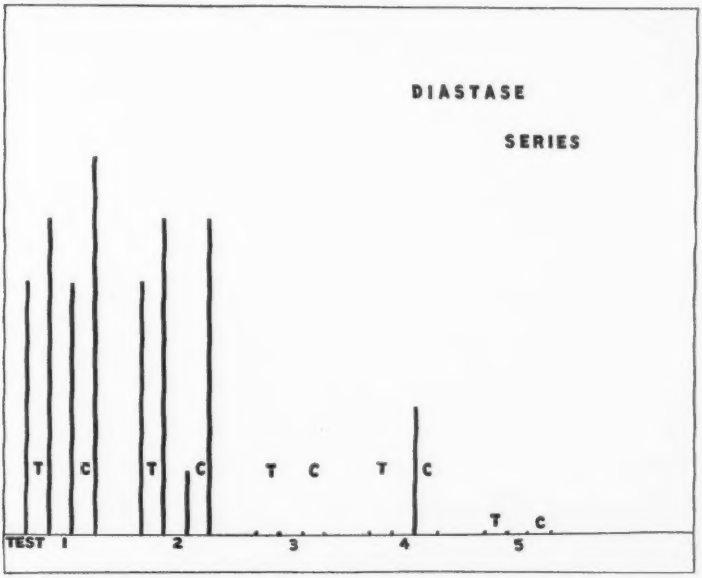


FIG. 8.—Time factor as related to starch digestion in presence of formaldehyde mixed 1–320 parts water: height of line indicates amount of starch remaining undigested (all conditions shown in duplicate); 1 cc. 0.5 per cent solution Merck's diastase, 10 cc. 25 per cent soluble starch solution, and 4 cc. formaldehyde used in tests (*T*), and a like amount of water in controls (*C*); time periods in hours tests 1 to 5 respectively, 1.25, 1.5, 1.75, 2, 2.5 hours.

plete in the controls in half that time, has also occurred in the presence of the formaldehyde. The series in the presence of 1–320 (fig. 8) formaldehyde was not so striking, but nevertheless shows satisfactorily that formaldehyde does not entirely inhibit the action of diastase, but retards the same. Turning to the effects on the starch digestion in living wheat of the concentrations of formaldehyde 1–320, 1–240, 1–160, and 1–80, a considerable quantity of

grain was treated to each concentration ten minutes, allowed to drain, stand moist for two hours, and then thoroughly air dried before an electric fan. The grain was then thoroughly ground in a mill, and extracts of 8 gm. lots made in 100 cc. of redistilled water. Ascending quantities of the water extract were then added to test-tubes each containing 5 cc. of soluble starch prepared as for the

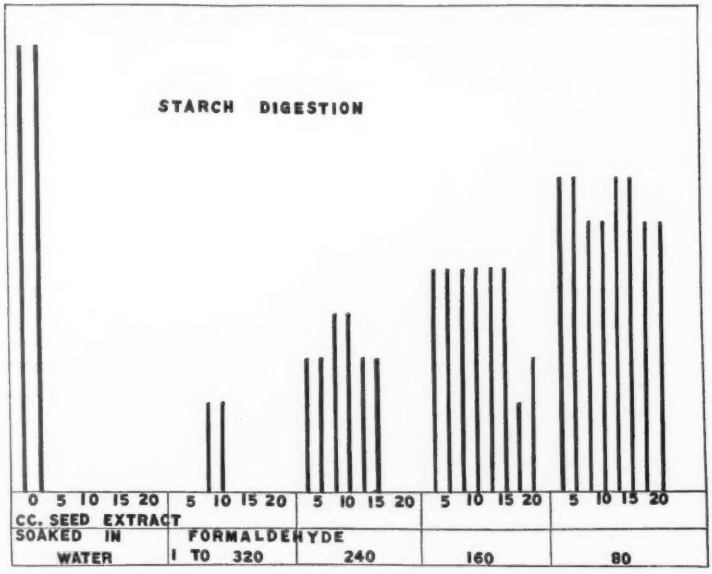

STARCH DIGESTION
0 5 10 15 20 5 10 15 20 5 10 15 20 5 10 15 20 5 10 15 20
CC. SEED EXTRACT
SOAKED IN WATER
FORMALDEHYDE
1 TO 320
240
160
80

FIG. 9.—Effect of formaldehyde treatment on diastatic activity of wheat extract, seeds treated 10 minutes; concentration of formaldehyde used in treatment varied, and also cubic centimeters of seed extract used: height of lines indicates amount of starch remaining undigested after incubation one hour at 40° C.; 5 cc. 25 per cent soluble starch solution used in each test (all conditions shown in duplicate).

other tests. After incubation for one hour at 40° C. complete digestion had occurred in the controls containing 5, 10, 15, and 20 cc. respectively of the extract of untreated seed. Fig. 9 shows that despite a few unexplained irregularities, the general trend is obviously a reduction in the amount of starch digestion, with a rise in the concentration of the formaldehyde originally used in treatment of the seed. This holds for 5, 10, 15, and 20 cc. seed extract tests

under all the conditions. From these data it would seem certain that treating wheat with formaldehyde retards the availability of carbohydrate to the germinating seedling. BOKORNY (12), reviewing the work of NEUBERG, in which variations showed in the inhibitory effects of different concentrations of formaldehyde on various enzymes and of the effects of the same concentration on different enzymes, explains differences in behavior on the theory of the molecular structure of the enzymes causing different linkages with the formaldehyde. Inasmuch as enzymes are commonly known to be associated at least with proteins, and as formaldehyde is known to react quantitatively with amino acids, as in the Sörensen titration, it is not surprising that effects of formaldehyde upon enzyme behavior should be observed.

AMINO ACIDS

In the light of the results of the tests on the diastatic activity effects of formaldehyde, it was thought well to determine the relationships to amino acids. Miss CHOATE (17) found amino acids occurring in ungerminated wheat and increasing in amount on germination. Miss ECKERSON (27) found only slight amounts of asparagine in the ripened grain, although histidine, leucine, asparagine, and arginine occurred during ripening. The chemical constitution of the wheat grain involves several linked amino acids according to OSBORNE (42), while ABDERHALDEN and SAMUELY (1) in a list of the amino acid constituents of gliadin of wheat flour give alanine, tyrosine, and glutamic acid as among those highest in amount.

As a preliminary test, known quantities of pure amino acids in water solution were determined by the Van Slyke method, both with and without the presence of varying amounts of formaldehyde. Alanine was secured from the organic laboratories of the Eastman Kodak Company, while glutamic acid hydrochloride and tyrosine were purchased from the Special Chemicals Company of Highland Park, Illinois. Careful checking through over 200 tests indicates that such linkages as are formed by formaldehyde and amino acids are broken by the Van Slyke process, and no reduction in nitrogen yields occurred incident to the presence of formaldehyde. The

common use of the formol titration in the determination of amino acids is based on our knowledge that such linkages do occur. It had been assumed that in case such a combination between formaldehyde and amino acids of the germinating seedling does occur the nitrogenous nutrition might easily be disturbed. It is very much hoped that the opportunity may be afforded later to check further upon this point, and also to determine the comparative amount of amino acids liberated in autolysis of treated and untreated seeds.

Respiration

Much effort has been expended in the determination of the effects of seed treatment upon the respiration. Peirce and co-workers (43) correlated germinative vigor with respiratory activity. Carbon dioxide has often been recognized as a measure of the activity of the metabolism in the tissues liberating the gas. It was desired to determine whether varying concentrations of formaldehyde, showing varying effects on viability, also affected carbon dioxide releasal in the same manner; in other words, whether the measure of formaldehyde injury may be had by the comparatively accurate carbon dioxide measurements.

Seed lots of 75 gm. each in duplicate were soaked in water as a check, and lots in duplicate in the varying concentrations of formaldehyde, period of soaking being ten minutes, after which they were drained and sealed in respiratory chambers submerged in a constant temperature bath at 28° C. for two hours before beginning the determination. Large museum jars were used for respiratory chambers, equipped with ground glass tops with openings for two-holed stoppers. The seeds were suspended on wire gauze six inches above the bottom of the chamber, while the tubing by which the gases were withdrawn from the chamber during the tests extended to the bottom of the jars. The water bath was 1.5 by 3 feet, and deep enough to permit the tall museum jars to be completely submerged in the water. Under the water bath were placed six porcelain resistance units connected to the lighting system of the laboratory. About 6 feet of small bore glass tubing was bent so as to be submerged in the bath, and filled with mercury, which served to conduct current from two gravity cells to a telegraphic

relay, which at the desired temperature turned off or on the heat under the bath. This arrangement permitted control of the temperature within 0.2° C. Careful checking of the temperature at different points in the bath indicated that stirring devices were not necessary, other than the convection currents from the bottom of the bath upward. Two chambers were used for water soaked wheat (controls), two for the treated wheat, and two blanks to permit checking against leakage.

After setting up the apparatus completely and before making a determination, each of the six complete trains was tested by suction as to its ability to hold up a column of mercury 10 inches high without small leaks permitting the column to settle back again. During the tests a gentle stream of air freed from carbon dioxide was drawn through each outfit for the entire period of hours of the run. In order that the rate of aeration might be uniform in the various outfits, and ample to provide for several complete changes of the air in the respiratory chambers during the course of the experiments, the suction secured from a water pump was conducted to the various chambers through tubing, connected to manometers in such a way that after careful calibration of the separate manometers, the rate of air flow could instantly be determined by a glance at the height of the paraffin oil surface in the manometers. Gas meters of this type were developed in connection with the chemical investigations incident to the recent gas warfare work, and are described in detail by BENTON (11). For each of the six trains air was drawn respectively through 50 per cent potassium hydroxide, a *U*-tube of moist soda lime, and through a barium hydroxide indicator to detect any failure of previous absorbents to remove all carbon dioxide. Air entered the respiratory chamber at the top and was removed from the bottom under the wheat arranged as described. The air then containing the carbon dioxide released by the wheat was drawn immediately through a bead tower containing fourth normal barium hydroxide, out and over another barium hydroxide indicator before passing to the tube connected to the water pump.

The amount of suction was regulated by ground glass stopcocks between the pump and the last indicator flask. With the six com-

plete trains to provide for, it was not deemed practicable to apply the type of automatic pipette arrangement used by BAILEY and GURJAR (9). Instead, a large bottle was thoroughly cleaned and aerated with carbon dioxide-free air and filled with the standard alkali. This reservoir was connected by tubing with a burette, and communication with outside air protected by soda lime traps. At the top of each of the bead tower columns was placed a separatory funnel guarded by a soda lime trap. Before each running the required number of cubic centimeters of the alkali were run directly into the separatory funnel previously washed free of carbon dioxide.

When the whole outfit was ready to make a running, carbon dioxide-free air was run for a sufficient period through the bead tower column to remove all carbon dioxide, before admitting the alkali from the separatory funnel directly into the bead column. This method of determination of carbon dioxide is essentially that described by TRUOG (54). Varying periods of aspiration in these measurements were employed, although experience showed that most satisfactory results could be obtained by employing a period of from four to five hours. No results were considered worthy of recording for second runs of any one lot of samples, as experience showed the need of extreme care to avoid introducing errors incident to the growth of saprophytes upon the check samples, particularly if they were retained at 28° C. longer than one day. BAILEY and GURJAR (9) allowed their moist wheat to stand several days before removing the stagnant air for carbon dioxide determination. Had they used the temperature of 28° C., and had their seed possessed a moisture content of 35–43 per cent, as was the case in these tests, it would have been impossible to avoid questioning the secondary factor of saprophytes which these experiments showed increased tremendously the output of carbon dioxide. Disregarding the fact that they failed entirely to keep their chambers aerated during the course of their work, however, it must be said that they incubated their seeds at 37.8° F., and worked with seeds of moisture contents much lower, in general between 12 and 20 per cent. NABOKICH (41) concluded that part of the carbon dioxide obtained in plant respiration is incident to the same microorganisms that vegetate on leaves and seeds. It was hence a source of much

concern to avoid any fluctuations in respiratory results incident to the gaseous exchanges of saprophytes which might easily be confused with the results of seed treatment. NABOKICH, however, determined that the respiration of microorganisms on seeds may be disregarded during the first day, counting from the time of wetting the seeds. It is thus believed that the data given here eliminate the errors incident to such secondary factor.

Throughout the work over forty runnings were made, representing over 160 different seed lots. As regards the possible criticism that the several per cent variation in moisture content based on dry weight might make the results incomparable, it must be borne in mind that absolute carbon dioxide yields of different runnings are not to be compared with each other, but only the four lots used in any one run. Careful analysis of the variations in carbon dioxide output as related to moisture content has indicated that these variations may not be ascribed to moisture content differences, these observations being made in duplicate independently for the four seed lots of any one running. Fig. 10 and table II summarize the entire results of the investigations on respiration. In all of the work care was taken to have present in the flasks and bead towers at least twice as much of the alkali as would be neutralized by the carbon dioxide liberated by the seeds during any one run. The graphs are expressed in terms of the percentages of the barium hydroxide neutralized. In each case 25 cc. of fourth normal barium hydroxide was used, and at the close titrated against fourth normal hydrochloric acid in the presence of phenolphthalein. If, for instance, 12.5 cc. was neutralized the graph would express 50 per cent values.

Fig. 10 shows marked depression of the respiratory rate for the highest concentrations (1–80) as compared with the water soaked controls. The depression of the respiration rate is evident, although decreasingly so, at 1–160, 1–240, and down to 1–320, the concentration usually used in seed treatment. At 1–400 and 1–1000 the difference between the controls and the treated samples was neither so great nor so constant as to indicate any marked effects of the formaldehyde on metabolism. Special care was used in checking out the situation at the 1–320 concentration, at which point

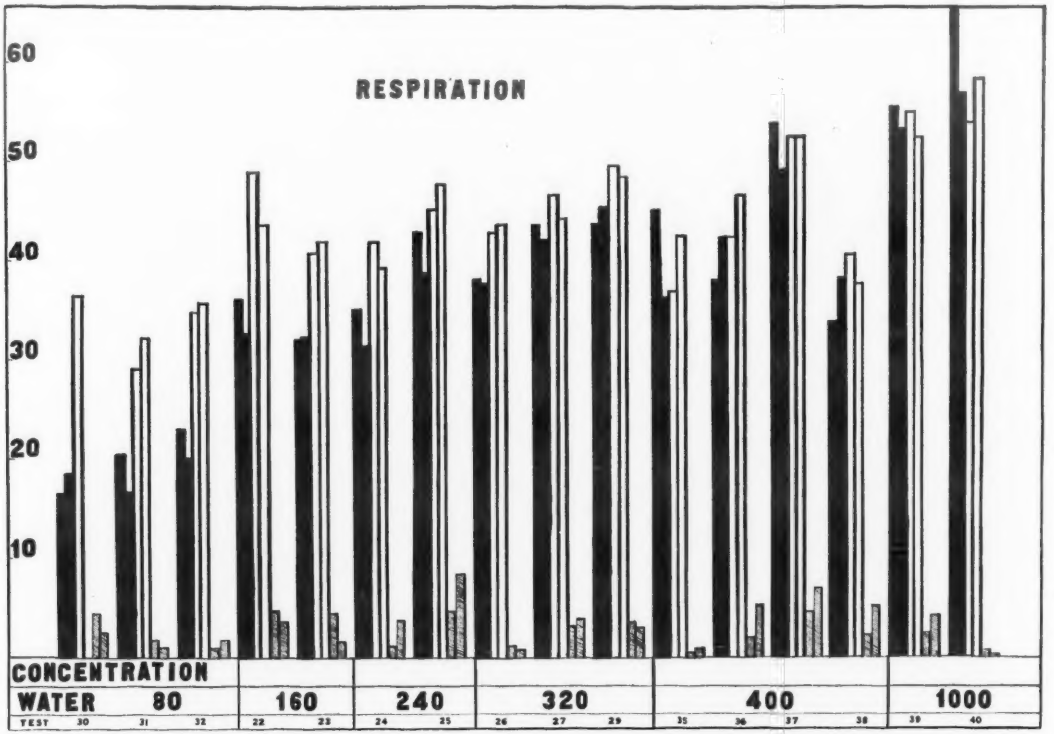


FIG. 10.—Respiration of wheat as influenced by treatment with formaldehyde of varying concentrations; results expressed in terms of percentage of neutralization of total number of cubic centimeters of alkali by carbon dioxide released by seeds; absorption in each case in 25 cc. N/4 barium hydroxide solution; six determinations for each test shown: solid black bars indicate carbon dioxide trapped from treated seeds, white bars indicate magnitudes for water-soaked control seeds, short bars with cross-lining indicate carbon dioxide from empty controls (error).

TABLE II

DATA ON RESPIRATION TESTS AS SHOWN IN FIG. 10

		Test	30	31	32	22	23	24	25	26	27	29	35	36	37	38	39	40
No. of test																		
Hours in respirometer before beginning test			2	2	24	2	2	2	2	2	2	2	5.5	2	2	2	3	24.5
Hours test			4.5	4	4	5	4	4.75	5	4.25	5.5	5	4.5	5	5	4	5.5	4
Hours seeds stood moist after treatment			2	2	2	2	2	2	2	2	2	2	5.5	2	2	2	3	24.5
Parts water to one part of formaldehyde			80	80	80	160	160	240	240	320	320	320	400	400	400	400	1000	1000
Treated wheat	Percentage moisture at end of test	1	40.15	38.85	38.85	.....	42.25	36.09	36.92	37.90	39.30	39.38	39.20	38.70	40.50	.....	43.60	43.60
		2	39.20	36.85	36.85	.....	40.23	35.25	37.70	39.70	38.80	37.60	35.65	36.35	38.80	.....	41.00	41.00
	Cc. barium hydroxide combined	1	4.10	5.11	5.72	8.95	7.97	8.95	10.59	9.45	10.77	10.81	11.16	9.44	13.36	8.48	14.02	16.33
		2	4.63	4.16	5.01	8.12	8.03	7.76	9.58	9.34	10.44	11.17	8.95	10.48	12.21	9.50	13.17	14.12
	Percentage cc. hydroxide combined	1	16.48	20.52	23.00	36.10	32.10	35.10	42.70	38.10	43.42	43.60	44.90	37.92	53.70	33.91	55.14	65.70
		2	18.60	16.72	20.14	32.72	32.40	31.30	38.65	37.62	42.10	45.10	36.10	42.20	49.10	38.20	53.00	56.80
Water-soaked control	Percentage moisture at end of test	1	.....	36.40	36.40	.....	41.60	36.40	37.00	41.00	37.30	37.80	37.88	39.90	38.40	.....	40.00	40.00
		2	37.40	36.85	36.85	.....	39.67	35.20	40.18	41.48	40.00	.....	36.60	42.28	39.48	.....	38.45	38.45
	Cc. barium hydroxide combined	1	.....	7.24	8.63	12.14	10.08	10.37	11.15	10.59	11.55	12.25	9.13	10.44	12.97	10.03	13.61	13.33
		2	9.03	8.01	8.91	10.75	10.40	9.72	11.80	10.82	11.14	11.98	10.53	11.51	13.01	9.33	12.98	14.41
	Percentage cc. hydroxide combined	1	.....	29.10	34.70	48.90	40.70	41.80	45.00	42.70	46.60	49.4	36.70	42.00	52.20	40.38	54.70	53.63
		2	36.30	32.20	35.80	43.4	41.90	39.2	47.6	43.6	44.95	48.40	42.40	46.30	52.30	37.5	52.20	58.00
Empty controls (error)	Cc. barium hydroxide combined	1	0.65	0.48	0.26	1.19	1.15	0.30	1.17	0.60	0.72	0.90	0.13	0.48	1.16	0.56	0.61	0.18
		2	1.13	0.28	0.46	0.92	0.42	0.95	2.12	0.20	1.00	0.78	0.08	1.33	1.76	1.31	1.06	0.08
	Percentage cc. hydroxide combined	1	2.61	1.93	1.05	4.80	4.64	1.21	4.72	1.14	2.90	3.63	0.52	1.93	4.66	2.25	2.45	0.72
		2	4.54	1.13	1.85	3.71	1.70	3.83	8.55	0.81	4.03	3.15	0.32	5.35	7.08	5.27	4.26	0.32

evidently the concentration is near the border line of injury, since 1–400 does not definitely display such uniform depression of respiration. It was desired to compare the respiratory rates of young seedlings from treated and untreated wheat, but as yet technique has not been devised which avoids the luxuriant development of *Rhizopus* in the warm moist atmosphere of the respirometer. In these tests, as in those with the seeds alone, special care was used to avoid air infection of the respirometers. Just previous to a running, the interior of each jar was wiped out with cotton moistened with mercuric chloride (1–1000). The further precaution was taken of flaming the gauze on which seeds and seedlings were placed. KARCHEVSKI, as quoted by BAILEY and GURJAR (9), found the energy of wheat respiration as measured by carbon dioxide releasal twelve times greater for the embryos than for the entire seeds. This seems to indicate that the data may be more largely influenced by factors affecting the embryos than otherwise. The facts that formaldehyde denatures proteins, that the embryo is rich in proteins, and that the respiration data show the effects of formaldehyde treatment, make it possible that the injurious effects of formaldehyde are intimately connected with injury to the embryo itself. This is in harmony with the findings of COLLINS (18), in a study of the coat of the barley grain, that the entry of solutions and hence the seat of selectivity is in the germ end of the grain.

It is of interest in this connection to note that although Miss HURD (33) believed the injurious effects of formaldehyde to be attained by a slow absorption of the gas liberated from paraformaldehyde, and although these studies of the penetration of formaldehyde do not show any sudden penetration of the fungicide, nevertheless within a period of time as short as three to six hours during respiratory determinations, some effect is exerted upon the seed which very definitely modifies the respiratory rate as compared with water soaked controls. One can but wonder whether here, as in the case of the studies of CROCKER and KNIGHT (24), we may not have in plant responses a more delicate indicator of injury than are the chemical reactions commonly used in detecting these injurious substances.

CATALASE

The work of recent years has shown that there often seems to be a relationship between respiration of plant tissues and the catalase content. APPLEMAN (4, 5) has shown this relationship in the case of potatoes and corn, CROCKER and HARRINGTON (23) in the case of seeds. The latter workers find that this relationship is not universal, for while imbibed Johnson grass has its respiration paralleled by the catalase activity, this is not true for the seeds of *Amaranthus*. The most interesting observation is further made that in the case of Johnson grass where this parallelism is found, neither the respiratory activity nor the catalase content is paralleled by the vitality of the seeds or the seedlings. Inasmuch as seed vitality and seedling vigor are definitely related to formaldehyde treatment, and this work has linked in also the effects upon respiration, it was thought to be of interest to determine whether any effects of seed treatment could be noted upon the catalase activity of wheat.

Catalase activity in wheat was determined much after the method suggested by APPLEMAN (2), and later employed with various modifications by other workers (17). Two series of experiments were conducted, one to see whether any effects of formaldehyde could be noted immediately after treatment while the grain was still moist, and another to see whether the effects of treatment persist on grain which has been treated and air dried before an electric fan in the laboratory and subsequently exposed for about a month to the air of the laboratory. The concentrations of formaldehyde used were 1–80, 1–160, and 1–320. About 1 gm. of air dried wheat was used in each case, weighed on the analytical balance, and results computed to the basis of 1 gm. For a reaction chamber a bottle of 250 cc. capacity was used and shaken continuously during the ten minutes of the test by a mechanical shaker making 129 excursions per minute. The reaction chamber was submerged in the constant temperature bath previously described, and was kept at 28° C. Dioxygen was used and neutralized with calcium carbonate. It was found that 3 mg. of the chemically pure salt used would neutralize 5 cc. of the peroxide, and this proportion was observed throughout. The gas evolved was run into a 100 cc. gas burette,

and all readings corrected for temperature and barometric pressure, the equivalent corrected volumes at 0° C. and 760 mm. being computed for 1 gm. sample. Ten cc. water and 5 cc. peroxide as neutralized were used.

In the case of the freshly treated seeds, they were soaked for ten minutes in the formaldehyde solutions, and kept moist for two or more hours, until tested. The moisture of the seeds precluded

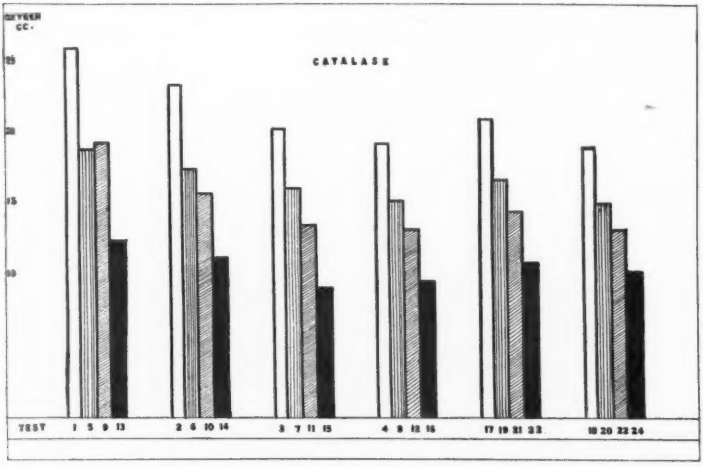


FIG. 11.—Effect of formaldehyde in varying concentrations upon ability of seed extract to liberate gaseous oxygen from dioxygen: height of lines indicates cubic centimeters of oxygen computed for 1 gm. samples and constant temperatures and pressures; extract from water-soaked seeds (controls) indicated by white, vertical lining indicates extracts from seeds treated in formaldehyde 1–320, diagonal lining formaldehyde 1–160, and solid black formaldehyde 1–80; in all cases seeds tested while still moist.

the possibility of passing the material through bolting cloth (**23**), but it was soon found that a material difference in the crop of oxygen given off resulted if the seed material was more or less glutinous and forming sticky masses, or dry enough to separate fairly well on grinding in a mortar with carborundum as an abrasive; hence all surface moisture was removed with filter paper before grinding up the seeds. Inasmuch as differing moisture contents of the differing lots would occur between the first lots tested and the last in a long series, the errors incident to this cause were avoided

as far as possible by testing consecutively the control seeds and those treated with 1–320, 1–160, and 1–80. Such a series of four naturally fall together for comparison in one group, and many such series were made, the combined results of which may easily be seen from fig. 11 and table III. With some small variations, the results were surprisingly uniform in showing the steady depressing effect of

TABLE III

DATA ON CATALASE TESTS AS SHOWN IN FIG. 11

Test no.	Concentration formalin	Weight of sample	Volume oxygen released	Barometer	Temperature	Hours between treatment and test	Corrected volume equivalent of 1 gm. sample
1	Water control	1.015	30.1	748	24	2	25.9
2	Water control	1.005	26.9	748	24	3.5	23.4
3	Water control	1.006	23.5	748	25	7	20.3
4	Water control	1.009	22.4	748	25	8.5	19.3
5	1–320	1.008	21.8	748	24	2.5	18.9
6	1–320	1.000	20.1	748	24	3.75	17.5
7	1–320	1.004	18.6	748	25	7.25	16.1
8	1–320	1.002	17.6	748	25	9	15.3
9	1–160	1.006	22.2	748	24	2.75	19.3
10	1–160	1.015	18.3	748	24	3	15.8
11	1–160	1.007	15.7	748	25	7.5	13.6
12	1–160	1.002	15.3	748	25	9	13.3
13	1–80	1.010	14.4	748	24	3	12.5
14	1–80	1.011	13.0	748	24	3	11.3
15	1–80	1.006	10.6	748	25	8	9.20
16	1–80	1.010	11.2	748	25	9.5	9.63
17	Water control	1.017	24.6	747	24.5	5	21.1
18	Water control	1.012	22.3	745.5	25	7.75	19.1
19	1–320	1.007	19.4	747	24.5	5	16.8
20	1–320	1.010	17.7	745.5	25	8.25	15.2
21	1–160	1.003	16.7	747	24.5	5.25	14.5
22	1–160	1.011	15.5	745.5	25	8.5	13.3
23	1–80	1.003	12.7	747	24.5	5.5	11.0
24	1–80	1.013	12.1	745.5	25	8.75	10.4

formaldehyde on catalase activity. As the concentration of the formaldehyde rose, the catalase activity as measured by oxygen yield fell.

In the studies of the effects of formaldehyde on catalase activity after the seeds had been dried about a month in the laboratory, comparison was made only between the control and the seeds treated in 1–80 formaldehyde. Fig. 12 and table IV show that there is a definite depressive effect of the treatment on catalase activity, but it is by no means so great as soon after treatment.

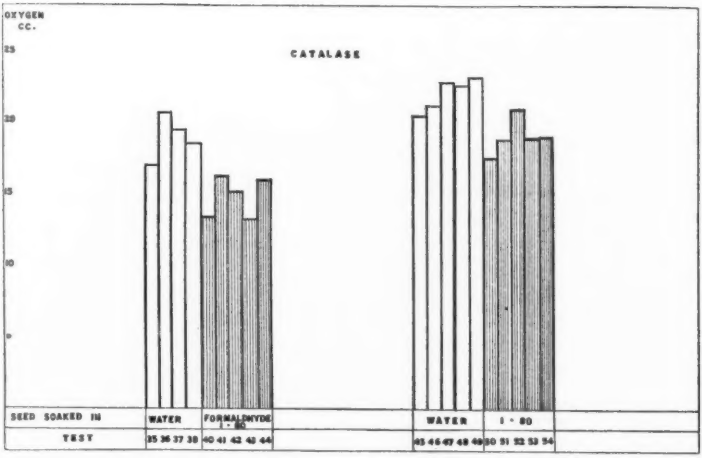


FIG. 12.—Effect of formaldehyde 1 to 80 upon ability of seed extract to liberate gaseous oxygen from dioxygen: height of lines indicates cubic centimeters of oxygen computed for 1 gm. samples and constant temperatures and pressures; extract of water soaked seeds (controls) indicated by white, treatment in 1–80 indicated by vertical ruling; in all cases seeds tested after thoroughly drying one month in laboratory after treatment.

TABLE IV

DATA ON CATALASE TESTS AS SHOWN IN FIG. 12

Test no.	Concentration formalin	Weight of sample	Oxygen (cc.)	Barometer	Temperature	Corrected volume equivalent of 1 gm. sample
35.....	Water control	1.014	20	743.5	25	17.10
36.....	Water control	1.002	24	743.5	25	20.7
37.....	Water control	1.005	22.5	743.5	23	19.5
38.....	Water control	1.006	21.5	743.5	24	18.6
40.....	1–80	1.014	15.7	743.5	24	13.5
41.....	1–80	1.005	18.8	743	23	16.3
42.....	1–80	1.002	17.5	743	23	15.2
43.....	1–80	1.020	15.5	743	23	13.3
44.....	1–80	1.000	18.5	743	23	16.1
45.....	Water control	1.011	23.5	745	21.5	20.5
46.....	Water control	1.011	24.2	745	20.5	21.2
47.....	Water control	1.012	26.1	745	20.5	22.9
48.....	Water control	1.014	25.9	745	21	22.6
49.....	Water control	1.014	26.7	745	21.5	23.2
50.....	1–80	1.006	20.0	745	21.5	17.6
51.....	1–80	1.003	21.3	745	20.5	18.8
52.....	1–80	1.002	23.8	745	21	21.0
53.....	1–80	1.011	21.8	745	21.5	19.0
54.....	1–80	1.001	21.7	745	21.5	19.1

This would seem to support the view that the injury is due more to exterior members retaining the formaldehyde which had been volatilized in part, than to a permanent injury to the embryo having resulted from the treatment.

General considerations

The treatment of seeds with fungicides is a process wherein one plant tissue (that of the parasite) must be destroyed, while another tissue (that of the seed) must be conserved. It is entirely probable that the points of fungicidal effectiveness and of danger to seeds are not far separated. DE ZEEUW (26), in noting this point, quotes work in which it was found that the seeds were more sensitive to formaldehyde than spores of either bacteria or fungi, when its action was deeper than the surface, as is essential to secure sterile seeds. He believes that the high concentrations of disinfectants required to care for the destruction of bacterial spores is explained by the protection afforded by the seed coat on which the spores are lodged. There is an interesting similarity between this statement and the findings of REIMER (46), who observed that in the control of the bacillus producing fire blight of pears, a disinfectant which is serviceable upon tools is ineffective when used upon the organic substrate of the wood of the tree itself.

It would seem that we have been too ready to jump to conclusions and give "recommendations" as to treatment upon the basis of germination data of more or less extent. In the light of the preceding results, it seems doubtful whether it is safe to postulate the boundary lines of safe and dangerous concentrations merely upon the basis of germination data. STEPHENS (50) has emphasized the relationship of seed treatment to subsequent lowered vitality of seedlings. It is thus entirely possible that concentrations which do not materially injure germination percentages do materially disturb the physiological processes related to germination and subsequent growth. Common agricultural practice and the findings of the War Emergency Board of American plant pathologists seem to indicate that 1–320 is at the edge of the danger zone, if indeed such zone is not here passed. If, as Miss HURD (33) believes, a polymer of formaldehyde is deposited on dried treated wheat, and subse-

quent injury to the grain is incident to the liberation of formaldehyde gas from the precipitate, with its resultant solution in the moisture content of the living cells, the process must be a slow cumulative one which is entirely in harmony with the definite although slow entry of formaldehyde herein shown actually to occur. Furthermore, even though the grain be treated with 1–320 formaldehyde, such a deposit of the polymers would result in the presentation to the living cells of a much stronger concentration than that of the dip used. Yet even at the concentration of 1–320 it is evident that while germination is often but lightly affected, the diastatic power of wheat is retarded, the catalases are less active, and respiration is definitely reduced. It is not impossible that such results indicate a decided tendency to a reduction in seedling vitality even in the presence of germination.

It is highly desirable that these studies be pursued further to determine the relationship of treatment to the proteolytic activities of the germinating seed, and further, to determine whether the recommended "presoak" of BRAUN (13) or the "post-washing" of Miss HURD (33) correspondingly modify the physiological activities and alleviate their injury.

Summary

1. Tests of formaldehyde entry into wheat have been made both by microchemical tests and by imbibitional studies, indicating that formaldehyde slowly enters through the seed coat.

2. Diastatic activity of the grain is retarded.

3. Respiration is slowed down.

4. Catalases are reduced in their ability to break down peroxides.

OREGON AGRICULTURAL EXPERIMENT STATION
CORVALLIS, ORE.

LITERATURE CITED

1. ABDERHALDEN, E., and SAMUELY, FRANZ, Die Zusammensetzung des "Gliadins" des Weizenmehls. Zeit. Physiol. Chem. **44**:276–283. 1905. Abs. in Jour. Chem. Soc. **88**:620. 1905.

2. APPLEMAN, C. O., Some observations on catalase. BOT. GAZ. **50**:182–192. 1910.

3. ———, Physiological behavior of enzymes and carbohydrate transformations in after-ripening of the potato tuber. BOT. GAZ. **52**:306–315. 1911.

4. APPLEMAN, C. O., Relation of catalase and oxidases to respiration in plants. Bull. 191, Md. Agric. Exp. Sta. pp. 16. 1915.
5. ———, Relation of oxidases and catalase to respiration in plants. Amer. Jour. Bot. **3**:223–233. 1916.
6. ARTHUR, J. C., Formalin for grain and potatoes. Ind. Agric. Exp. Sta. Bull. **77**:38–44. 1900.
7. ATWOOD, W. M., A physiological study of the germination of *Avena fatua*. BOT. GAZ. **57**:386–414. 1914.
8. AUERBACH, F., and BARSCHALL, H., Studies of formaldehyde. II. The solid polymers of formaldehyde. Arb. Kais. Gesundheitsamt. **27**:183–230. 1908. Abs. in Chem. Abs. **2**:1125. 1908.
9. BAILEY, C. H., and GURJAR, A. M., Respiration of stored wheat. Jour. Agric. Res. **12**:685–715. 1918.
10. BAKKE, A. L., and PLAGGE, H. H., Studies on the absorption and germination of wheat treated with formaldehyde. Ia. Acad. Sci. **26**:365–375. 1919.
11. BENTON, A. F., Gas flow meters for small rates of flow. Jour. Ind. and Eng. Chem. **11**:623–629. 1919.
12. BOKORNY, T., Bindung des Formaldehyds durch Enzyme. Biochem. Zeitschr. **94**:69–77. 1919. Abs. in Bot. Abs. **8**:641.
13. BRAUN, HARRY, Presoak method of seed treatment. Jour. Agric. Res. **19**:363–392. 1920.
14. BRITTLEBANK, C. C., The effect of formalin and copper sulphate solution on the germination of wheat. Jour. Dept. Agric. Victoria **11**:473–476. 1913.
15. BROWN, ADRIAN J., On the existence of a semipermeable membrane inclosing the seeds of some of the Gramineae. Ann. Botany **21**:79–87. 1907.
16. BURMESTER, H., Comparative investigations on the effect of various methods of seed treatment on the germination of seed. Zeitschr. Pflanzenkrank. **18**:154–187. 1908.
17. CHOATE, HELEN A., Chemical changes in wheat during germination. BOT. GAZ. **71**:409–425. 1921.
18. COLLINS, E. J., The structure of the integumentary system of the barley grain in relation to the localized water absorption and semi-permeability. Ann. Botany **32**:381–414. 1918.
19. COONS, G. H., and MCKINNEY, H. H., Formaldehyde injury to wheat. 21st Mich. Acad. Sci. Rept. 321–324. 1919.
20. CRANEFIELD, F., The influence of formalin on the germination of oats. Wis. Sta. Rept. 327–335. 1901.
21. ———, The influence of formaldehyde on the germination of oats. Wis. Sta. Rept. 268–272. 1902.
22. ———, Treatment of seed oats for the prevention of smut. Wis. Sta. Rept. 363–367. 1903.

23. CROCKER, WILLIAM, and HARRINGTON, GEORGE T., Catalase and oxidase content of seeds in relation to their dormancy, age, vitality, and respiration. Jour. Agric. Res. **15**:137–174. 1918.

24. CROCKER, WILLIAM, and KNIGHT, LEE I., Effect of illuminating gas and ethylene upon flowering carnations. BOT. GAZ. **46**:259–276. 1908.

25. DELEPINE, MARCEL, Formaldehyde in gaseous liquid and solid states and in solution. Bull. Sci. Pharmacol. **16**:146–160. 1910. Rev. in Chem. Zentr. 1909 (I). 1467.

26. DE ZEEUW, RICHARD, The comparative viability of seeds, fungi, and bacteria when subjected to various chemical agents. Centralb. Bakt. 2 Abt. **31**:4–23. 1912.

27. ECKERSON, SOPHIA H., Microchemical studies in the progressive development of the wheat plant. Bull. 139, Agric. Exp. Sta. Wash. 1917.

28. GUARCO and JEROME, Preventing polymerization of formaldehyde. Rev. in Chem. Abs. **3**:2202. 1909.

29. HAAS, PAUL, and HILL, T. G., An introduction to the chemistry of plant products. Longmans Green & Co. Citation page 149.

30. HAYWOOD, J. K., and SMITH, B. H., A study of the hydrogen peroxide method of determining formaldehyde. Jour. Amer. Chem. Soc. **27**:1183–1188. 1905.

31. HEALD, F. D., and WOOLMAN, H. M., Bunt or stinking smut of wheat. Bull. 128, Agric. Exp. Sta. Wash. pp. 24. 1915.

32. HENDERSON, L. F., Experiments with wheat and oats for smut. Bull. 53, Agric. Exp. Sta. Idaho, pp. 15. 1905.

33. HURD, ANNIE MAY, Injury to seed wheat resulting from drying after disinfection with formaldehyde. Jour. Agric. Res. **20**:209–244. 1920.

34. ———, Seed coat injury and viability of seeds of wheat and barley as factors in susceptibility to molds and fungicides. Jour. Agric. Res. **21**: 99–122. 1921.

35. JOHNSON, A. G., STAKMAN, E. C., and POTTER, ALDEN A., Preliminary review of results on seed injury tests with standard disinfectants. Report from Office of Cereal Investigations, U.S. Bur. Pl. Ind. 1918.

36. KIESSLING, L., Experiments on the germ ripening of grain. Landw. Jahrb. Bayern **1**:449–514. 1911.

37. MACKIE, W. W., Progress report on cereal smuts. Smut control measures practised in California. 1918 (unpublished).

38. MCALPINE, D., Effect of formalin and bluestone on germination of seed wheat. Bull. 12, Dept. Agric. So. Aust. pp. 21. 1908.

39. MELHUS, I. E., A quick method of eliminating seed borne organisms of grain. Science N.S. **50**:21. 1919.

40. MUELLER, H., and MOLZ, E., Experiments on the combating of stinking smut in winter wheat by means of the formaldehyde treatment. Fühlings Landw. Z. **63**:742–752. 1914. Rev. in Chem. Abs. **9**:2122. 1915.

41. NABOKICH, A. J., Über den Einfluss der Sterilization der Samen auf die Atmung. Ber. Deutsch. Bot. Gesells. **21**:279–291. 1903.
42. OSBORNE, THOMAS B., The proteins of the wheat kernel. Carnegie Inst. Publ. no. 84. pp. 119. 1907.
43. PEIRCE, G. J., DARSIE, M. L., and ELLIOTT, C., A study of the germinating power of seeds. BOT. GAZ. **58**:101–136. 1914.
44. PERDRIX, L., Bactericidal action of dry formaldehyde. Ann. Inst. Pasteur **29**:701–719. 1908.
45. PICCHIO, G., On the germination of wheat treated with some fungicides and insecticides. Coltivatore **59**:435–439. 1913. Rev. in Exp. Sta. Rec. **30**: 837. 1914.
46. REIMER, F. C., Unpublished work at the Southern Oregon Branch Station, Talent, Oregon.
47. SCHROEDER, H., The resistance of wheat and barley grains to poisons with special reference to seed sterilization. Centralb. Bakt. 2 Abt. **28**:492–505. 1910.
48. SHERMAN, H. C., KENDALL, E. C., and CLARK, E. D., Studies on amylases. I. An examination of methods for the determination of diastatic power. Jour. Amer. Chem. Soc. **32**:1073–1086. 1910.
49. SHULL, C. A., Semipermeability of seed coats. BOT. GAZ. **56**:169–199. 1913.
50. STEPHENS, D. E., Unpublished annual reports from the Sherman County Dry Farm Branch Station, Moro, Oregon.
51. STEVENS, F. L., Experiments upon the effect of formalin on the germination of oats. N.C. Agric. Exp. Sta. Rept. 30–36. 1908.
52. STEWART, R., and STEPHENS, J., The effect of formalin on the vitality of seed grain. Bull. 108, Agric. Exp. Sta. Utah, 145–156. 1910.
53. THOMAS, CECIL C., Seed disinfection by formaldehyde vapor. Jour. Agric. Res. **17**:33–39. 1919.
54. TRUOG, E., Methods for the determination of carbon dioxide and a new form of absorption tower adapted to the titrametric method. Jour. Ind. and Eng. Chem. **7**:1045–1049. 1915.
55. WALLDÉN, J. N., Damage caused by threshing of wheat and rye and its influence upon sensibility to treatment with fungicides and to storage. Sveriges Utsädesför. Tidskr. pp. 24. 1917. Rev. transl. from Dept. Agric.
56. ———, Experiments on the prevention of stinking smut on winter wheat. Sveriges Utsädesför. Tidskr. **22**:242–252. 1912. Rev. in Exp. Sta. Rec. **28**:242. 1913.
57. WINDISCH, R., Concerning the effect of formic aldehyde on germination. Landw. Vers. Stat. **49**:223–226. 1897. Rev. in Exp. Sta. Rec. **9**:955. 1898.
58. ———, On the action of formaldehyde on germination. Landw. Vers. Stat. **55**:241–252. 1901. Rev. in Exp. Sta. Rec. **13**:656. 1902.

DEVELOPMENT OF THE GEOGLOSSACEAE

G. H. DUFF

(WITH PLATES VIII–XII)

Introduction

Detailed knowledge of the ontogeny of Helvellinean fungi had its beginnings in the work of DITTRICH (7), whose paper on the complete development of *Mitrula phalloides* and *Leotia gelatinosa* (*L. lubrica*), with observations on other forms, has been the starting point for all subsequent life history studies in this group. This worker found that in the early stages of growth the ascocarps of *Mitrula* and *Leotia* possess a veil which, although evanescent, covers over the hymenium during the early part of their history, and thus renders their development endogenous. Heretofore the Helvellineae had been regarded as an essentially exogenous order, and SCHROETER (20) had separated them on this basis from the Pezizineae, in which group the hymenium was considered to be developed characteristically in a closed cavity which later opened. DURAND (11) confirmed and extended DITTRICH'S observations in the introduction to his excellent monograph of the Geoglossaceae. He states that he had observed the veil of *Mitrula phalloides* before learning of DITTRICH'S work. He had, in addition, noted the occurrence of a particularly well organized veil in *Microglossum viride*, and the conspicuous envelopes of *Spathularia velutipes* and *Cudonia lutea*. Of the latter he published beautiful photographs, illustrating these structures macroscopically.

The next contribution to this subject was a paper by MCCUBBIN (17) on the development of *Helvella elastica*. His work constitutes the first study in the family Helvellaceae, all the previous work having been confined to the Geoglossaceae. Here also it is stated that a membrane incloses the ascocarp in the younger stages of the form with which he worked. It is disorganized relatively early, and the last traces of it are cast off with the appearance of the paraphyses. BROWN (4) follows with an account of the development

of the ascocarp of *Leotia*, but, strange to say, he makes no reference to a veil, although his material apparently included very early stages of this form. His failure to mention the veil might be due to the fact that he was chiefly concerned with the origin of the asci and the nuclear phenomena connected therewith.

From these investigations it will be seen that in two of the three Helvellinean families, the Geoglossaceae and the Helvellaceae, an endogenous origin of the hymenium has been claimed. Until the publications of FITZPATRICK (**14, 15**), nothing was known of the conditions prevailing among the members of the remaining family, the Rhizinaceae. FITZPATRICK has now shown quite conclusively that *Rhizina undulata*, the type of the family, possesses no investment at any stage of its history, and that, therefore, not only is the hymenium "exposed from the first," but the ascocarp itself is naked, that is, "gymnocarpous."

In a preliminary communication the writer (**10**) briefly outlined his findings in an examination of four Geoglossaceous forms, namely, *Cudonia lutea*, *Spathularia velutipes*, *Trichoglossum hirsutum*, and *Leotia lubrica*. The veils of *Cudonia* and *Spathularia* were found to be present practically from the first. *Trichoglossum* proved to be devoid of all traces of a veil at every stage. On the other hand, *Leotia lubrica* showed some slight traces of a veil, which might be comparable with the one claimed for it by DITTRICH, but the series of stages which the writer had under observation did not include ones sufficiently young to make diagnosis positive, and the question must still be regarded as an open one.

Turning to the question of the sexuality of the Helvellineae, there is still less to record. DITTRICH found no highly organized sexual apparatus in either *Mitrula* or *Leotia*, and he characterizes both forms as apogamous. The fertile hyphae here are differentiated from the vegetative threads at an early stage as conspicuous, deeply staining cells, with large single nuclei.

Similarly MCCUBBIN describes and figures the multinucleate, fertile elements of *Helvella* as having their origin in the vegetative hyphae, from which they spring at a later stage than is the case with *Mitrula* and *Leotia*. No sign of any body resembling an ascogonium was discovered at any stage.

On the other hand, BROWN (4) concludes that *Leotia* possesses an ascogonium which makes its appearance early in the history of the ascocarp. From this the fertile hyphae arise, and may be seen in succeeding stages, passing upward into the cap, where after much branching they reach the hymenium and form asci. This ascogonium was seen in but one plant, and was observed only in an empty and partly degenerated condition. The manifestations of sexuality in *Leotia*, therefore, cannot be said to have been established beyond the possibility of a doubt.

It is only in the work of FITZPATRICK (15) that we find a wholly satisfactory description of sexuality in any of the Helvellineae. This worker has given a very careful and detailed account of sexuality in *Rhizina*. In this form, while the ascocarp primordia are yet small, certain multicellular hyphae near the center are transformed into procarps. This transformation entails a great increase in diameter and in the number of nuclei in the cells, the assumption of an irregular coiling habit, and the development of a somewhat elongated terminal cell which may be considered a trichogyne. No antheridia are present, and the trichogyne is functionless. When maturity is reached, the centrally situated cells of the procarp proliferate ascogenous hyphae, and into these the nuclei from all parts of the procarp migrate, passing from cell to cell through pores which have previously been formed in the transverse septa. These nuclei migrate in pairs. Evidence of nuclear fusions in the ascogonial cells or in the ascogenous hyphae is entirely lacking, and neither conjugate nor simple divisions were observed to occur in the latter.

The conditions obtaining in the species discussed in this paper have already been summarized (10). It was pointed out that no evidence of the existence of any type of procarp bodies has been found in *Trichoglossum*, in which the ascogenous hyphae arise from threads of vegetative form adjacent to the hymenium. In *Cudonia* and *Spathularia* the structures interpreted as procarps arise at a comparatively late stage in the development of the ascocarp from threads differentiated while the fructification is yet very young. They are numerous, more or less irregular in form, and while in *Cudonia* very distinct trichogynes are organized, similar structures are absent from *Spathularia*. In *Cudonia* the cells of the procarp appear to be uninucleate at first, later becoming multinucleate,

while in *Spathularia* they are multinucleate from the beginning. The nuclei in the later stages of these bodies are arranged in pairs. Ascogenous hyphae take their origin in these procarps, and after engaging in crozier formation, give rise to asci.

Materials and methods

The material for this investigation was all obtained by collection, and was variously fixed, the fluids of Flemming and Carnoy, medium chrom-acetic acid and picro-sublimate, being used. It was imbedded in paraffin and cut in serial sections 2.5–10 μ thick. The usual staining methods were employed. Safranin and acid fuchsin proved to be the best general purpose stains, while Haidenhain's haematoxylin gave much the best results where details were required. In some cases the young fructifications sectioned *in situ* were stained by VAUGHAN'S (**21**) method for the differentiation of fungous hyphae and host tissues. This method gave very clear differentiation of the fungous tissue from the substratum, but had the serious disadvantage of impermanence.

As is not infrequently the case, the cells of certain tissues, while in a state of rapid growth and development, proved to be exceedingly difficult to differentiate cytologically, due to the density with which they are filled with deeply staining substances. As a result it has been impossible to answer some questions of nuclear behavior as fully as would be desirable. Otherwise the tissues appeared to be quite easily amenable to the usual microtechnical methods.

As already indicated, this paper deals in more or less detail with the four forms *Cudonia lutea*, *Spathularia velutipes*, *Trichoglossum hirsutum*, and *Leotia lubrica*. With the object of emphasizing the comparative aspects of the investigation, the contents are arranged in two parts, the first of which deals with the development of the ascocarp, with particular reference to the veil, and the second with the manifestations of sexuality in these plants.

I. Development of ascocarp

CUDONIA LUTEA

The earliest stage of *Cudonia lutea* of which we have knowledge is shown in fig. 3. At this stage the ascocarp is very minute, and measured but 84 μ from base to apex. It can be seen to consist of

loosely interwoven hyphae, which are obviously mycelial elements elevated to their present position chiefly by the upward growth of a central knot of conspicuous, deeply staining threads. The latter are of peculiar interest on account of the part they play in the development of the fertile system. At first sight, because of their marked differentiation from the other threads composing the young fructification, they might easily be taken for ascogenous hyphae, or even for ascogonia. At a later stage (fig. 6) the proliferations of these threads give rise to bodies of characteristic form and history, bodies that admit of one interpretation only, namely, that they are procarps. The first appearing threads must therefore have some significance other than this.

Clearly differentiated bodies are known to originate the procarps or corresponding bodies in various Ascomycetes. In *Ascobolus carbonarius* (DODGE 8) the procarps arise through the germination of asexual conidia borne on special mycelial branches. BROWN (3) found that in *Xylaria tentaculata* the Woronin hyphae are formed by the continued modification of threads that are differentiated at the center of the perithecium anlage while the latter is very young and deeply imbedded in the stroma. According to Miss DAWSON (6), a well differentiated thread appears in *Poronia punctata*, which at a later stage grows into a procarp.

The nearest approach to the first differentiated hyphae of *Cudonia* is to be found, however, in the disco-lichens. NIENBURG (18) gives a description of the development of the fruit bodies of four of these lichens, *Usnea barbata*, *Baeomyces roseus*, *Sphyridium byssoides*, and *Icmadophila aeruginosa*. It appears from his account that more or less well developed carpogonia arise in these forms, not from the ordinary hyphae of the ascomata in which they are found, but from threads which are differentiated at a considerably earlier stage. To these threads NIENBURG applied the name "Primordialhyphen" or "generativen Hyphen." In *Icmadophila* they appear before the fruit body has begun to rise above the surface of the thallus.

NIENBURG apparently was not very logical in his use of the terms "Primordialhyphen" and "generativen Hyphen." He employed the first in his study of *Usnea*, and the latter in studies of *Bae-*

omyces, *Sphyridium*, and *Icmadophila*. He drew no distinction between the so-called "Primordialhyphen" of *Usnea* and the "generativen Hyphen" of *Sphyridium* and *Icmadophila*. In *Baeomyces*, however, the carpogonia are so poorly defined that they are not separable from the "Primordialhyphen," so to the entire system in *Baeomyces* he extended the term "generativen Hyphen." Then, because of the evidently close natural relationship of *Sphyridium*, *Icmadophila*, and *Baeomyces*, he adopted the designation "generativen Hyphen" in connection with these forms. It is to be understood, therefore, that the "generativen Hyphen" of *Baeomyces* are not exactly homologous with those of *Sphyridium* and *Icmadophila*.

In the preliminary paper (10) the writer adopted the term "generative hyphae" rather than "primordial hyphae" to designate the pre-fertile threads of *Cudonia* and *Spathularia*. This was done because the Geoglossaceous forms appear to be more closely related to *Baeomyces*, *Sphyridium*, and *Icmadophila* than to *Usnea*. The occurrence of such a system of threads acting as precursors to the procarps is unusual, so far as the writer is aware, being found only in the disco-lichens, and so may be considered of sufficient importance to warrant emphasis.

The second developmental stage (fig. 4) shows a very definitely organized outer tissue, which has apparently been thrown up by the mycelial threads. By following through the series (figs. 4–7) this tissue may be seen to be identical with that later regarded as the veil. The veil in *Cudonia*, therefore, originates at a time when the ascocarp is nothing more than a cushion of mycelial threads with a core of generative hyphae, and can therefore be said to be present from the first. It is to be noted that at this stage a well marked clear zone separates the generative threads from the veil tissue. This clear zone contains only the remnants of the mycelial hyphae that once surrounded the generative threads. Fig. 5 illustrates the next developmental stage of *Cudonia*. Here the generative hyphae form an elongated mass of filaments of which the oldest are at the lower, and the newly formed elements, recently proliferated from them, are at the upper extremity. This upward growth of the generative threads has completely obliterated at the top the

clear space that once surrounded them on three sides, and at this point they are now in a subapical position, almost in contact with the veil tissue. Remnants of the clear space still remain on both sides, but it is rapidly being filled by the growth of fundamental tissue which has arisen from the mycelium, and which has pushed up before it the mass of generative hyphae. The intrusion of fundamental tissue from below has its beginning at an earlier stage, and indications of it may be seen on both sides at the base of the ascocarp in fig. 4. Judging from these two stages, it would appear that the most rapid formation of new tissue, at least during the early stages, takes place at the base rather than at the apex of the ascocarp.

The subapical position of the generative hyphae is retained as the ascocarp increases in size, until the apex broadens out to form the cap. These hyphae then pass into the cap, and there some of their branches are transformed into procarps. This transformation entails an increase in diameter, the assumption of an irregular coiling habit, and the development of a process which passes from the procarp more or less directly through the veil to the exterior. These processes are to be regarded as trichogynes. The history of the procarps will be discussed in greater detail subsequently. While at this stage, the upper portions of the cap are densely filled with generative hyphae, procarp complexes, and their proliferations the ascogenous hyphae, interwoven in an almost inextricable tangle (fig. 6).

It is important to note that until this time no sign of a hymenium has made its appearance. Very shortly after paraphysis formation commences, however, and following this the ascogenous hyphae proceed with the proliferation of asci. Paraphyses in *Cudonia* arise from undifferentiated vegetative elements. They are multiseptate, in this respect differing from the description given for this species by DURAND, and their cells are multinucleate. At first they are straight, but later become strongly recurved at the tips, which are also somewhat enlarged.

The veil still completely invests the entire ascocarp, including the hymenium. Over the hymenium it has become separated from the tissue of the subhymenial region, with, indeed, no attachment at all except at the edges (fig. 7). Nevertheless it does not cease

growth, but persists without rupturing, and keeps pace with the enlargement of the cap for considerable time. This power of independent growth is undoubtedly responsible for the persistence of the veil in this form, and is expressive of the distinct nature of the veil as an organ of the ascocarp and of its high degree of organization. Dehiscence finally takes place, by which time many of the asci are matured and their spores ready to be discharged. In the meantime, with the advent of the paraphyses the trichogynes disappear. The remaining portions of the procarps are visible for some time after, with cells empty or containing only a few strands and knots of plasm, evidently in a disorganized condition (figs. 12, 41). Finally practically all traces of them disappear.

Spathularia velutipes

Fig. 14 is a section of the earliest stage of *Spathularia velutipes* that has been studied. It can be seen to have been growing upon much rotted deciduous wood, and to have protruded above the substratum for somewhat less than 0.5 mm. From its size and organization it is evident that it represents a corresponding stage considerably older than the earliest *Cudonia* plants here figured. The interior of the young fruit body is composed of undifferentiated fundamental tissue, which is surrounded by a compact and well defined layer, which, as in *Cudonia*, is the investing membrane or veil.

By the use of suitable counter stains the veil of *Spathularia velutipes* may be sharply differentiated into two layers, an inner and an outer, the former compact and comparatively thin walled, and the latter looser and thicker walled. As the ascocarp increases in size, the outermost layer is split by tension into small adherent cell masses, which to the naked eye give the fruit body a velvety appearance. The power of independent growth is just as much a characteristic of the very persistent veil of *Spathularia velutipes* as it is of the membrane of *Cudonia*. In contrast to the condition of *Cudonia* at this stage, however, the interior of the ascocarp is still undifferentiated, and no threads corresponding to the generative hyphae of that form are yet visible.

By the time the ascocarp has attained the stage represented in fig. 15, conspicuous hyphae have made their appearance, and are situated as the generative hyphae come to be in *Cudonia*, just

behind the apex. These conspicuous threads in the young *Spathularia* plant are also generative hyphae, for, as will shortly be seen, certain of their proliferations at a very much later stage are transformed into procarps. As the young ascocarp increases in size the pointed apex becomes broadened out, and the generative hyphae are distributed in an arch below it. This arch widens in concert with the gradual expansion of the apex into a somewhat globular cap, which as time goes on assumes the spatular shape characteristic of the mature plants of the genus (figs. 16, 17).

The expansion of the apex into the cap is marked by the appearance of paraphyses. The paraphyses of *Spathularia* arise from vegetative hyphal threads, the end cells of which become somewhat club-shaped. Various students of other species, as McCUBBIN (**17**) and BROWN (**4**), have found paraphyses arising from specialized storage cells. This does not occur here, the cells from which the paraphyses arise being insufficiently differentiated to be regarded as storage bodies. In form, septation, and nucleation the paraphyses of *Spathularia* resemble those of *Cudonia*.

While the paraphyses are still young, procarps become evident for the first time. They are scattered about in irregular fashion through those portions of the cap containing the generative hyphae, and are complexes conspicuous for their size and staining qualities (figs. 19–21, 43–45). An examination of all stages after this until the veil is shed and spores are being cast reveals their presence continuously. They vary in shape; some appear as coiled structures, some are immensely swollen, and others take the form of chains of cells. These bodies arise from the generative hyphae just as was the case in *Cudonia*, and from them ascogenous hyphae proceed directly to the hymenium, where in turn they may be seen giving rise to asci. Further growth results, as in the case of *Cudonia*, in the introduction of no new tissues or organs, but in the mere expansion of the ascocarp. The hymenium reaches a fairly well advanced state of maturity before the veil bursts. The observations of the writer have not established any regularity in the method of rupture, but DURAND states that "the veil seems to rupture by a crack running around the plant just above the stem," and exhibits photographs which illustrate his point.

Trichoglossum hirsutum

The very young fructification of *Trichoglossum hirsutum* shown in fig. 22 measures 175 μ in height, and is of simpler structure than the corresponding stages of either *Cudonia* or *Spathularia*. It is composed of compactly intertwining threads, the walls of which are comparatively thick and very dark. The hyphae all appear to be alike except for some, which, situated near the periphery and at right angles to it, extend beyond the ascocarp as straight, sharp pointed, unicellular hairs of greater diameter and thicker walls than the other threads. These setae correspond to similar ones described by Brown in *Lachnea scutellata* (5), and by Fitzpatrick in *Rhizina undulata* (14). The setae of *Trichoglossum* are differentiated somewhat earlier than in *Rhizina*, but do not contain a glutinous substance such as is found in those of the latter form. The setae in *Trichoglossum* appear to be continuously produced over all peripheral parts of the ascocarp as it grows, but those that are earlier formed persist for some time, and come to be imbedded deeply in the new tissues that grow up around them. This is particularly evident in the hymenial region, where such growth is rapid (figs. 25, 26).

Paraphysis formation commences reatively early at the apex of the ascocarp, before it has begun to broaden out to form the cap (figs. 24, 25). The paraphyses are large, unbranched, multiseptate threads, the cells of which are usually uninucleate, but apparently may come to contain several nuclei in exceptional cases. They are strongly coiled at the tips.

Shortly after the paraphyses are organized, broadening of the apex of the ascocarp begins, and the hymenium extends over the entire surface of the globular cap as it takes shape and expands into the mature condition.

From the figures it may be seen that the hymenium is of undoubtedly exogenous origin, and, moreover, the ascocarp itself is clearly gymnocarpous. Throughout the entire series of stages careful examination has failed to reveal the presence of an investment of any kind. *Trichoglossum hirsutum*, therefore, adds one more species to those Helvellineae the hymenium of which is exposed from the first and the fructification gymnocarpous.

The vegetative threads in a well developed ascocarp of *Trichoglossum hirsutum* constitute a loosely interwoven tissue in which the individual cells of the threads are clearly distinguishable, even in thick sections. The threads pass with more or less uniformity from the base to the apex of the ascocarp. Since they are thick walled and consist of uninucleate cells, it is not difficult to see that hyphae of any other type are absent. The paraphyses make their appearance at an early stage (fig. 24), but ascus formation does not commence until the cap is well shaped. Then, just beneath the bases of the paraphyses in the subhymenium, there appear thin walled hyphae which, unlike the vegetative threads, form a very densely interlaced tissue. These constitute the ascogenous system of *Trichoglossum*. It is certain that no bodies such as the generative hyphae and procarps of *Cudonia* or *Spathularia* stand out as specific organs, nor is it possible to distinguish the homologues of procarps as distinct from the ascogenous hyphae.

The ascogenous hyphae are short, since they are differentiated in such close proximity to the hymenium. Asci arise from them in the usual manner. The formation of an ascus is preceded by the inversion of the tip of an ascogenous hypha to form a crozier, the ascus growing out of the penultimate cell. The asci are very large, with dense protoplasm, and, especially after the dark multiseptate spores are mature, form very conspicuous objects in the hymenium (fig. 26).

LEOTIA LUBRICA

Since material of this form had been collected, and since previous investigators are not in complete agreement on two important points in the life history, it was thought advisable to examine the material with a view to the possibility of shedding some further light on these points. The questions to which reference is made are those of the presence of the veil and the origin of the ascogenous hyphae, and attention was confined to these questions alone.

Two specimens at approximately the same well advanced stage showed irregular tissue fragments overlying parts of the ascocarp and the hymenium (figs. 27, 28). These specimens were not the youngest of the fruit bodies examined. In no others, however, whether younger or older than these, were any similar evidences

seen. These fragments are composed of interwoven threads more or less degenerated in appearance, and in one case still connected with one another by a few hyphae, which give them the appearance of having been torn apart by the growth of the ascocarp (fig. 27). DITTRICH (7) describes the veil of *Leotia* as having a ground substance of gelatinous material formed by the progressive inward swelling ("Verquellung") of the peripheral hyphae. In this matrix are imbedded threads which are rather compactly interwoven. Apparently, therefore, so far as structure is concerned, the tissue fragments observed correspond fairly well to the veil tissue of *Leotia* as described by DITTRICH. Had very young ascocarps been available, and had such tissue been found covering them, particularly as entire structures, DITTRICH'S view that *Leotia* is at first angiocarpous would have been substantiated. On the other hand, had the material included a satisfactory series of very young stages which did not show traces of a veil, the writer would have felt justified in denying its presence in *Leotia lubrica*. The youngest stages available, however, were advanced sufficiently to show a well differentiated cap and young paraphyses, and consequently were considerably older than the youngest stages which BROWN, and probably also DITTRICH, had under observation. Under these circumstances the writer does not feel that the evidence is sufficient to lend conclusive support to either view. It would seem best, therefore, to regard the question as still open.

SIGNIFICANCE OF VEIL IN HELVELLINEAE

Ever since the appearance of DITTRICH'S (7) original paper on this subject, each succeeding investigation has served to emphasize the inadequacy of the distinction drawn by SCHROETER (20) between the Helvellineae and the Pezizineae. According to this classification, in the former group the hymenium is formed upon the surface of the ascocarp, and therefore is freely exposed from the first, while in the latter the hymenium is originally inclosed. SCHROETER'S fundamental idea appears to have been that in the Pezizas the hymenium is formed within the ascocarp in an inclosed depression, while in the Helvellas it appears upon a flat or convex surface, not closed in.

The discovery of the veil introduces a new morphological feature not contemplated in SCHROETER'S scheme. The value of the veil as an important taxonomic criterion depends, not so much upon the fact that it may at first inclose the hymenium, as upon its nature as an organ of the ascocarp. If it were simply the roof of a depression, comparable with the roof that covers over the young hymenium of a *Peziza*, and which breaks away to form the edge of the cup, then the Helvellinean forms in which it occurs would plainly be only modified Pezizas. But if, as seems to be the case, it were a distinct envelope which covers over the entire ascocarp, and perhaps incidentally also the young hymenium, then so far as we know it is a feature not represented in the Pezizineae, with the possible exception of the as yet uninvestigated Helotiaceae and Mollisiaceae.

In reviewing the conditions prevailing in the Helvellineae, it is found that the veil is absent in the only member of the Rhizinaceae which has been studied, it is present in the only member of the Helvellaceae of which we have knowledge, while at the present time the Geoglossaceae are about equally divided. In the last named family the following species may be considered as angiocarpous: *Mitrula phalloides*, *Leotia lubrica* (DITTRICH 7); *Microglossum viride* (DURAND 11); *Cudonia lutea*, *Spathularia velutipes* (DURAND 11, DUFF 10). The gymnocarpous members appear to be *Geoglossum glabrum*, *G. difforme*, *Trichoglossum velutipes* (DURAND 11); and *Trichoglossum hirsutum* (MCCUBBIN 17, DUFF 10). It may be noted that the gymnocarpous species are all closely related, belonging to genera that were at one time comprised in the single genus *Geoglossum*, while the others, with the exception of *Microglossum viride*, belong to less closely related genera. This means, so far as our evidence goes, that the *Rhizina* and *Geoglossum* groups are typically gymnocarpous, while the remaining Helvellineae are angiocarpous.

In searching for a structure in other Ascomycetes which might be homologized with the Helvellinean veil, we find one in the *Baeomyces* group of the disco-lichens. Some such relationship had already suggested itself to DITTRICH, who, after emphasizing other points of similarity, goes on to say that in *Baeomyces* the upper portions of the thallus which overlie the anlage of the fruit body, and which later surround the young fructification, seem to assume the

"functions" of a veil. Although he does not say so explicitly, it is a fair inference that DITTRICH considered this thalloid investment of the young *Baeomyces* fructification and the inclosing membranes of *Mitrula* and of *Leotia* homologous, for he implicitly adduces this as evidence of the Geoglossacean affinities of *Baeomyces*. DITTRICH's conclusions were based upon the description of the development of the fructification of this lichen given by KRABBE (16). NIENBURG (18) re-examined more recently some of the forms with which KRABBE worked, and the results recorded in his paper present even more interesting parallelisms to the conditions prevailing among some of the Geoglossaceae.

In *Icmadophila aeruginosa* the covering of the ascocarp is more distinct and more persistent than in *Baeomyces*. Figs. 1 and 2, taken from NIENBURG, represent sections of very young fruits of the former species. Here it may plainly be seen that the superficial layer of the thallus grows into an envelope for the young ascocarp and incloses it until it has attained considerable size, after which the growth of the ascocarp becomes too rapid for the extension of the epithecial tissue, which then ruptures. The fructification does not subsequently become covered with any other tissue which might be compared to a veil, and the hymenium therefore develops exogenously. As has already been indicated, however, the point of importance in this connection is not the origin of the hymenium, but only the occurrence of an envelope which incloses the entire ascocarp, and which constitutes a morphologically distinct organ of the fruit body. In the opinion of the writer, therefore, the envelopes of *Baeomyces* and *Icmadophila* are strictly comparable and homologous with those of the Geoglossaceae. From this it follows that in any natural scheme of classification some lichens, at least those of the *Baeomyces* group, must be brought in with the angiocarpous Geoglossaceous forms, as also possibly some members of the Helotiaceae.

II. Sexuality

CUDONIA LUTEA

It is in the youngest stages of *Cudonia lutea* that we find the beginnings of the hyphal system destined finally to give rise to asci. As we have seen, here there are differentiated from the

mycelium certain threads which have been called generative hyphae, and which are the most prominent central components of the simply organized fructification. These threads are clearly differentiated in the stained preparation from the rest of the ascocarp. So vigorously do they react to stains that all details of cell structure are obscured, even when nuclear stains are applied.

The generative hyphae grow upward as the ascocarp increases in length, remaining subapically situated. When the upper part of the fruit body is differentiated into the cap these hyphae make their way into it, and in that position certain branches of this system appear that are enlarged, irregularly coiled, and deeply staining (fig. 6). These are the procarps. The procarp coils are continued upward by processes which can only be regarded as trichogynes (figs. 6, 8, 9). These are multiseptate and follow a more or less direct course toward the exterior. Where the procarps lie in a suitable position the trichogynes may be followed for their whole length, and may be seen to penetrate the veil tissue and to reach the exterior. Sometimes the procarps are deeply imbedded, however, and in these cases it is impossible to follow the trichogynes as far as the surface of the ascocarp.

The number of procarps formed in this manner in a single fruit body appears to be very large, although it is not possible to count them, on account of the way in which they are interlaced and because of the presence of masses of generative and ascogenous hyphae. Some idea of their number may be conveyed, however, when it is known that as many as seven distinct trichogynes have been counted in a single longitudinal section 10 μ thick through a region of the cap in which they are numerous. Their distribution is irregular. Some of them are formed closely under, or even partly imbedded in the veil tissue, while others are situated at a distance from the surface in the direction of the center of the cap (figs. 6, 8, 9). Sometimes they are localized in one or two restricted portions of the cap, being entirely absent from others.

Fig. 11 is a portion of an unusually loosely organized procarp. This section does not contain any of the upper portion of the procarp, but it shows most of the lower part and the generative hypha from which it arose. The latter follows a somewhat

straighter course than is usual with these threads, and hence its relation to the coil is rendered very obvious. The generative hypha is more deeply stained than the surrounding vegetative threads, while the procarp coil itself is quite opaque. Figs. 32–35 represent other procarp bodies at approximately the same stage of development as that of fig. 11. Most of these figures are taken from sections cut longitudinally through the ascocarp, but fig. 34 represents portions of a procarp found in two contiguous sections of a series taken in a transverse plane. These illustrations give some idea of the diversity of form displayed by these structures. They vary from an almost straight, slightly twisted series of cells, to coils such as that of fig. 11. These illustrations also show the relationships of the procarps and trichogynes. This relationship is evident in fig. 6. Two procarps and their trichogynes appearing in this section are shown enlarged in fig. 8, where, however, on account of the thickness of the section and the meandering habit of the threads of the procarp and trichogynes, not all of these structures can be obtained in sharp focus at the same time. The protrusion of the trichogyne from the surface of the ascocarp is shown well in fig. 13, which is the first section of a series through the cap of an ascocarp at this stage. The exposed tip of a trichogyne, cut off with the first section, is clearly shown, several of its cells being visible.

No structures have been found that could be regarded as spermogonia, and no bodies that correspond to spermatia. Furthermore, the writer has no evidence that the trichogynes functioned in any way, and since they disappear very soon, is inclined to regard them as vestigial organs in *Cudonia*.

As may be seen from the illustrations, the procarps are very deeply staining bodies, and in their earlier stages it is impossible to differentiate them cytologically. Nevertheless here and there an occasional cell may be seen to contain what is doubtless a single nucleus, while a smaller number contain two (figs. 32–35). Thus what slight evidence there is points to the original condition of these bodies as being uninucleate. A little later on, however, they become multinucleate. This takes place soon after the appearance of the paraphyses, and is preceded by the disappearance of the trichogynes. At this stage the remaining cells of the procarps become

more distended, and their contents do not stain quite so densely, and thus cell structures can be distinguished with a greater degree of certainty. How the originally uninucleate cells become multinucleate is not known. The nuclei are small, and are frequently unequal in size. They often appear to lie in pairs (figs. 36, 37, 39, 40).

While at the multinucleate stage, or even before they can be seen definitely to be multinucleate, the procarp cells form branches which bud out from them (figs. 36, 38, 40). These branches are ascogenous hyphae, and can be recognized directly as such from the fact that in well advanced specimens in which the formation of asci is just beginning they have been seen passing from procarp complexes, which are usually empty and degenerated at this stage, to the hymenium, and there giving rise to asci (fig. 10). Ascogenous hyphae may be seen passing from an old procarp complex to the hymenium, which is just being differentiated (fig. 10). In the subhymenium they are in direct connection with cells undergoing crozier formation preparatory to the formation of asci. The cells of the ascogenous hyphae are usually multinucleate, but may be binucleate.

The proliferation of one or more asci from an ascogenous hypha by the formation of a crozier, with its four nuclei and various evolutions, has been described by numerous workers, and need not be detailed again. It will be sufficient to say that in *Cudonia* the penultimate cell usually grows out into an ascus, but a succession of croziers may be formed, and there may be a fusion of the ultimate and the antepenultimate cells, followed by the formation of an ascus or of another crozier. Several croziers may be formed from a single cell of an ascogenous hypha, and when all these are confined to the distal end of the cell a peculiar candelabra-like arrangement of asci results.

The two nuclei which find their way into the young ascus fuse at once. The fusion nucleus enlarges as the ascus grows, and finally assumes very large proportions. Divisions of the nucleus begin when the ascus has reached about half its ultimate size, and these divisions succeed one another very rapidly. When eight daughter nuclei have been formed the spores are delimited.

Spathularia velutipes

The origin of the generative hyphae in *Spathularia* has already been described. Mention has also been made of the fact that they come to occupy such a position in the ascocarp that when the hymenium has been formed they will lie just beneath it. Apart from their somewhat larger size, conspicuous staining qualities, and restricted position in the ascocarp, the generative threads do not appear to differ from the vegetative hyphae. The cells of both contain from one to several nuclei.

After the paraphyses have been formed, procarps grow out from some of the cells of the generative hyphae. The procarps are of relatively large proportions and become very strongly chromophilous. They are curious conspicuous complexes of hyphae. Attention has already been called to them, and some of them are illustrated in figs. 19–21 and 43–45. They appear to exhibit no uniformity of structure, but their cells are all multinucleate, and frequently distinct pairing of nuclei is observable (figs. 43–45.)

Ascogenous hyphae arise from these procarps. This is very easily demonstrated where the hyphal complex lies particularly close beneath the hymenium, and where the ascogenous hyphae pass directly into the hymenium. Under these circumstances the ascogenous hyphae take on something of the staining qualities of the cells from which they arise, and they may be traced with ease into the hymenium, and there may be seen in connection with asci. Fig. 21 is a plexus of such ascogenous hyphae arising from a group of large cells, some of which are visible. This large group may be traced through a series of sections 7.5 μ in thickness. The origin and termination of these threads are not visible in all sections, but by following through the series their connections in both directions are easily ascertained.

Fig. 19 affords another illustration of this type, showing two enlarged procarp cells, somewhat depleted of their contents, one containing a single nucleus and the other two nuclei. From these cells deeply staining ascogenous hyphae are to be seen passing outward, following a very irregular course to the hymenium, and there in direct union with young asci. The group of uninucleate asci of about the same age shown in this figure appears to have arisen

from ascogenous hyphae originating in the same procarp cells. More frequently, however, the hyphae passing from the procarps do not retain their staining qualities sufficiently long to make it possible to follow them to the hymenium, especially when they are much branched or when they follow a devious route. The cells of the ascogenous hyphae are multinucleate, and the nuclei appear to be paired (fig. 45).

McCUBBIN, who described bodies in the ascoma of *Helvella elastica* which bear considerable resemblance to those at present under discussion, took the view that they are merely storage organs. In addition to ascogenous hyphae McCUBBIN claims to have found paraphyses arising from these so-called storage cells. Judging from his figures, however, the paraphyses arising in this manner are so far from typical that it may be questioned whether they are actually paraphyses. Since the "storage bodies" of *Helvella* resemble the procarps of *Spathularia* in being multinucleate, in having the nuclei arranged in pairs, and in producing ascogenous hyphae, it would seem to the writer that they might also be interpreted as sex organs. FITZPATRICK (15) has already expressed this opinion. He states that "several significant facts would seem to indicate that at least part of these 'storage bodies' constitute some type of sexual apparatus, particularly the statement that they are sometimes found giving rise to ascogenous hyphae." The ascogenous hyphae of *Spathularia* behave as in the case of *Cudonia* in the evolution of asci. The description given for the latter form may be taken to include them both.

LEOTIA LUBRICA

According to DITTRICH, the fertile threads of *Leotia lubrica* are differentiated from vegetative hyphae at an early stage in the history of the ascocarp. While they do not appear to be easily traced in intermediate stages, they become conspicuous again as maturity approaches, this time in the cap, where, lying just beneath the hymenium, they give rise to asci. Although DITTRICH does not figure the youngest stage in which they are visible, as he does in the case of *Mitrula phalloides*, he says that these two forms are much the same in this regard. If so, the fertile hyphae appear at a very early stage, indeed, one almost comparable with the youngest *Cudonia* plants described in this paper.

On the other hand, BROWN (4) has figured the remains of what he interpreted to be an ascogonium, a structure found by him in one case only, at the base of an older ascocarp than the youngest with which DITTRICH presumably worked. The ascogenous hyphae pass up from the ascogonium, and may be traced to the cap, where they become much branched, finally finding their way to the hymenium. The writer's material did not include stages young enough to make possible a direct confirmation of either of these statements, but it did show hyphal complexes just previous to the formation of the first asci strikingly suggestive of those already described for *Spathularia*.

At this period the ascogenous hyphae appear under the hymenium as deeply staining threads in various stages of crozier formation and ascus proliferation, and since they are not numerous as yet, they are easily distinguished and recognized. Beneath them in the cap may be seen at the same time very conspicuous groups of greatly enlarged cells. Some of these enlarged cells are quite empty, some contain light vacuolated protoplasm, and others are densely filled with deeply staining contents, identical in appearance with those of the ascogenous hyphae. These enlarged cells are usually rounded, occurring singly or in groups, but they may assume any of a great variety of forms. Their distribution in the ascocarp follows no regular arrangement whatever. They contain from one to several nuclei which are variable in size and sometimes may be very large (figs. 47–53). From these large cells ascogenous hyphae arise. This is readily demonstrated wherever they occur close to the hymenial region.

BROWN has described what he regards as storage cells in the ascocarp of *Leotia*. Of these he says: "While the hymenium is being differentiated some of the vegetative hyphae give rise to large storage cells. . . . . These large storage cells are formed in rows and give rise to paraphyses. The storage cells are at first multinucleate, but the nuclei usually fuse as growth proceeds." He affirms that the nuclei of these cells, where they are more than one, may be very unequal in size, and the fusion nuclei are sometimes of extraordinarily irregular form. The writer has not been able to find storage cells which exactly correspond to those described by BROWN. The only large unusual cells noted were those

already described, and it is believed that these are BROWN'S "storage cells." Careful attention was given to the possibility of paraphyses arising from these cells, but in the many sections examined for the purpose of confirming this observation, not a single clear case of the origin of paraphyses in these bodies was found.

The fact that ascogenous hyphae arise from these special cells in *Leotia* makes it necessary to consider the possibility that they represent some form of sex apparatus. Obviously, before this interpretation can actually be put upon them, it will be necessary to re-examine stages comparable with the youngest which BROWN studied in order to make sure whether or not an ascogonium occurs at the base of the young ascocarp. If there be no ascogonium there, *Leotia* would then resemble *Spathularia* in its manifestation of sexuality.

SEXUALITY IN HELVELLINEAE

The phenomena of sexuality in the Helvellineae bring forward some interesting questions. In the first place, the large number of procarps occurring in these Geoglossaceous forms is noteworthy; the overwhelming majority of Discomycetes possess but a single one. FITZPATRICK (15) drew attention to this point in his discussion of *Rhizina undulata*, which form is characterized by the production of several of these bodies. This investigator also indicated that while this character tends to separate the Helvellineae from other Discomycetes proper, it brings them closer to the disco-lichens, which are notable for their compound apothecia. FITZPATRICK quotes a number of lichen forms of which this is true, and to these may be added those which constitute the subject of NIENBURG'S (18) researches, namely, *Usnea barbata*, *Baeomyces roseus*, *Sphyridium byssoides*, and *Icmadophila aeruginosa*. It may be mentioned also that in those species in which the procarp is not obviously greatly reduced, for example in *Rhizina undulata* and in *Cudonia lutea*, it is of the same general form as that which characterizes lichens, that is, it consists of a modified, more or less coiled multicellular hypha the terminal portion of which constitutes a trichogyne. The trichogyne is unicellular in *Rhizina* and multicellular in *Cudonia*.

Another feature in common with the lichens is the fact that the procarps of the Helvellineae are not "initial organs" arising from the mycelium and later becoming surrounded by the tissue of the ascocarp, as they are in *Ascobolus*, etc., but are formed within an already well developed ascocarp. This is true of *Rhizina*, in which the fructification begins as a "wholly undifferentiated button of mycelium." It is not until the ascocarp has attained a diameter of about 1 mm. that the procarps appear. They then arise by the differentiation of certain centrally situated hyphae of the fruit body. In *Cudonia* the procarps arise at an even later stage, that is, after the ascocarp has become differentiated into cap and stem, while those of *Spathularia* are delayed still further in their appearance.

There is still another point of resemblance between these Geoglossaceous fungi and the disco-lichens in their common possession of a feature noted by NIENBURG (18), namely, that the procarps are offshoots of a unique hyphal system (designated by him "generativen Hyphen"), which makes its appearance at an early stage. NIENBURG shows that in *Icmadophila aeruginosa* the "generativen Hyphen" appear as deeply staining threads in the young ascocarp anlage before it becomes erumpent. As the fructification increases in size these hyphae proliferate to form scattered knots of threads, which are connected together, having a common origin, by "Verbindungshyphen." The latter are evidently a part of the system of "generativen Hyphen." These knots become transformed into procarps, and distinct trichogynes are formed which penetrate the tissues of the ascocarp and project into the air. Although spermatia frequently become attached to the trichogynes there is no conclusive evidence that they are functional, or that a process of fertilization takes place. Many of the procarps disorganize without producing ascogenous hyphae, but those that survive act as the source of these threads.

In *Sphyridium* the first elements of the pre-fertile system, the "generativen Hyphen," appear at a somewhat later stage than in *Icmadophila*. In this form they grow into a series of very clearly defined nests of hyphae, which, as in *Icmadophila*, are connnected together by "Verbindungshyphen." The growth of the vegetative

hyphae carries these knots to the upper portion of the ascocarp near the surface and separates them from one another. The ascogenous hyphae arise from these structures. They evidently represent some form of sex apparatus, but because of their lack of conventional trichogynes and their irregular habit, NIENBURG hesitates to give them the full status of typical "carpogones." He says: "Ich glaube deshalb, dasz regelrechte Trichogyne bei Sphyridium nicht mehr angelegt werden, sondern dasz wir hier reduzierte Gebilde vor uns haben, die wahrscheinlich früher als Empfängnisapparate gedient, heute aber diese Funktion aufgegeben haben."

In *Baeomyces* the young fructification is sunken in the thallus and is distinguished as a mass of hyphae more closely interwoven than those of the thallus. At the center of this mass appear certain enlarged, deeply staining threads which are the "generativen Hyphen," and which are the source of the ascogenous hyphae. NIENBURG'S uncertainty in the interpretation of these bodies is indicated in the following quotations: "So ist zu vermuten, dasz auch hier auf sehr frühem Stadium, Askogone als Verzweigungen der vegetativen Hyphen gebildet werden, die man nur mit unsern Mitteln von den übrigen Gewebe—dem askogenen wie dem vegetativen—nicht deutlich unterscheiden kann. . . . . Vielleicht sind die dunklen Hyphen in den Knäueln der Fig. 17 solche Askogone, vielleicht sind es aber auch schon askogene Hyphen." He decides that they may represent carpogonia, but if so, that they are very much reduced in *Baeomyces*, which he regards as an apogamous form. It is to be noted, however, that ascogenous hyphae still arise from these bodies even if they are structurally reduced.

These brief reviews indicate how close is the correspondence in the history of the fertile system between some of the disco-lichens and of the Geoglossaceae. In both groups the procarps are numerous; they have, in general, the same type of structure; and in both are formed at a more or less advanced stage from a system of threads which makes its first appearance while the ascocarp is very young.

It is interesting that in both groups parallel series are found showing comparable stages in structural reduction. In the lichens we have a graded series in *Icmadophila*, *Sphyridium*, and *Baeomyces*.

In *Icmadophila* the procarps are slightly if at all reduced, in *Sphyridium* they have lost the trichogyne and become somewhat irregular in form, while in *Baeomyces* it is questionable whether such structures can be said to occur at all. Among the Geoglossaceae we have *Cudonia* illustrating a condition entirely comparable with that of *Icmadophila*, while *Spathularia* resembles *Sphyridium* in the much-reduced nature of its procarps. In *Trichoglossum* the sex organs do not stand out as morphologically distinct structures. That these stages in reduction give a picture of the direction of evolution of sexuality in both groups is the opinion of the writer. Beginning with a form of procarp in which a true process of fertilization through the medium of the trichogyne took place, the evolution of these plants seems to have been marked by the successive suppression of the male organ, the trichogyne, and finally the procarp as a distinctive morphological body.

The facts observed in the Geoglossaceous forms examined point in a contrary direction to the opinion held by BLACKMAN and WELSFORD (1), as stated in their paper on *Polystigma rubrum*. They maintain that in all cases where a degenerate ascogonium occurs, the ascogenous hyphae will probably be found to originate independently of this organ. Their own recorded observation that the well organized procarp of *Polystigma rubrum* takes no part whatever in the formation of ascogenous hyphae lacks confirmation. Indeed, NIENBURG (19), in a more recent work on this species, maintains that ascogenous hyphae do originate in the procarp. BROOKS'S (2) similar contention with regard to the part played by the procarp of *Gnomonia erythrostoma*, and FISCH'S (13) statement on Woronin's hypha in *Xylaria polymorpha*, are offset by H. B. BROWN'S (3) observation that the cells of the Woronin hypha of *X. tentaculata* actually do become ascogenic.

The parallelism shown to exist between Geoglossaceous and disco-lichen forms must surely be significant of phylogenetic relationship. The question of the relationship of these groups is of practical interest, in view of the conviction on the part of certain botanists that the lichen genera should be distributed among those of the fungi proper. It is recognized by many who favor such a procedure that the history of the reproductive tracts must ulti-

mately be the basis of any such reclassification. The following quotation from FINK (12) exemplifies this view: "The classification of the fungi is really based in the main upon the morphological relationships of the reproductive areas, and in the lichens also these relationships surely have a greater importance in classification than the form-relationships of the thallus." This being so, such correspondences as have been disclosed here should indicate a basis of relationship between the Geoglossaceae and the *Baeomyces* group of the disco-lichens. This evidence of relationship is made stronger by the homology shown to exist between the veils which inclose the ascocarp in many of these two groups. The further extension of this relationship should aid materially in the problem of classification, and should give direction to our ideas concerning the affinities of these two interesting groups of plants.

Summary

1. The development of the ascocarp in *Cudonia lutea* and in *Spathularia velutipes* is notable for the appearance of an inclosing membrane which covers over the entire fructification, and which constitutes a morphologically distinct organ of the fruit body. Very early stages of *Cudonia* show its presence, and it is one of the first organs of the ascocarp to be differentiated. *Trichoglossum hirsutum* lacks entirely any such investment, and the ascocarp is therefore gymnocarpous. *Leotia lubrica* shows some evidences of possessing an evanescent veil, but this is a point which requires further elucidation.

2. The value of the Helvellinean veil as a systematic criterion depends more upon the fact that it is a morphologically distinct organ inclosing the entire ascocarp than that it may result in an endogenous development of the hymenium. This being so, the closest homology to the Helvellinean veil is to be found, not in the Pezizineae, but in the *Baeomyces* group of the disco-lichens. This is adduced as evidence of relationship between these groups.

3. The fertile systems of *Cudonia lutea* and *Spathularia velutipes* appear at an early stage in the history of the fructification as threads which have been called "generative hyphae." These hyphae proliferate with the other tissues of the ascocarp, and at

a later stage give rise to the procarps. The procarps of *Cudonia* are irregularly coiled, and are provided with multiseptate trichogynes which pass outward and protrude through the veil. The nuclear history is not fully known, but the original condition of the cells of the procarp seems to be uninucleate, later becoming multinucleate, and at this stage giving evidence of nuclear pairing. Ascogenous hyphae arise from the cells of the procarps, after which the procarps become emptied of their contents and finally disappear. The procarps of *Spathularia* appear somewhat later than in *Cudonia*, and are formed close to the hymenium. They are irregular, do not possess trichogynes, and are obviously reduced in structure. They are multinucleate throughout their history, and the nuclei are paired. Ascogenous hyphae arise from them.

4. In *Trichoglossum hirsutum* there is no structural differentiation of sex organs. The ascogenous hyphae arise from threads which do not differ in form from vegetative ones.

5. In *Leotia lubrica* bodies resembling in some respects the "storage bodies" described by BROWN for the same form were found to produce ascogenous hyphae. On this account it is suggested that they may represent sex organs, that is, they may be degenerate procarps.

6. It is pointed out that there is a very close correspondence in the history of the fertile systems of the Geoglossaceae and of certain disco-lichens of the *Baeomyces* group. This is cited as further evidence of relationship between them.

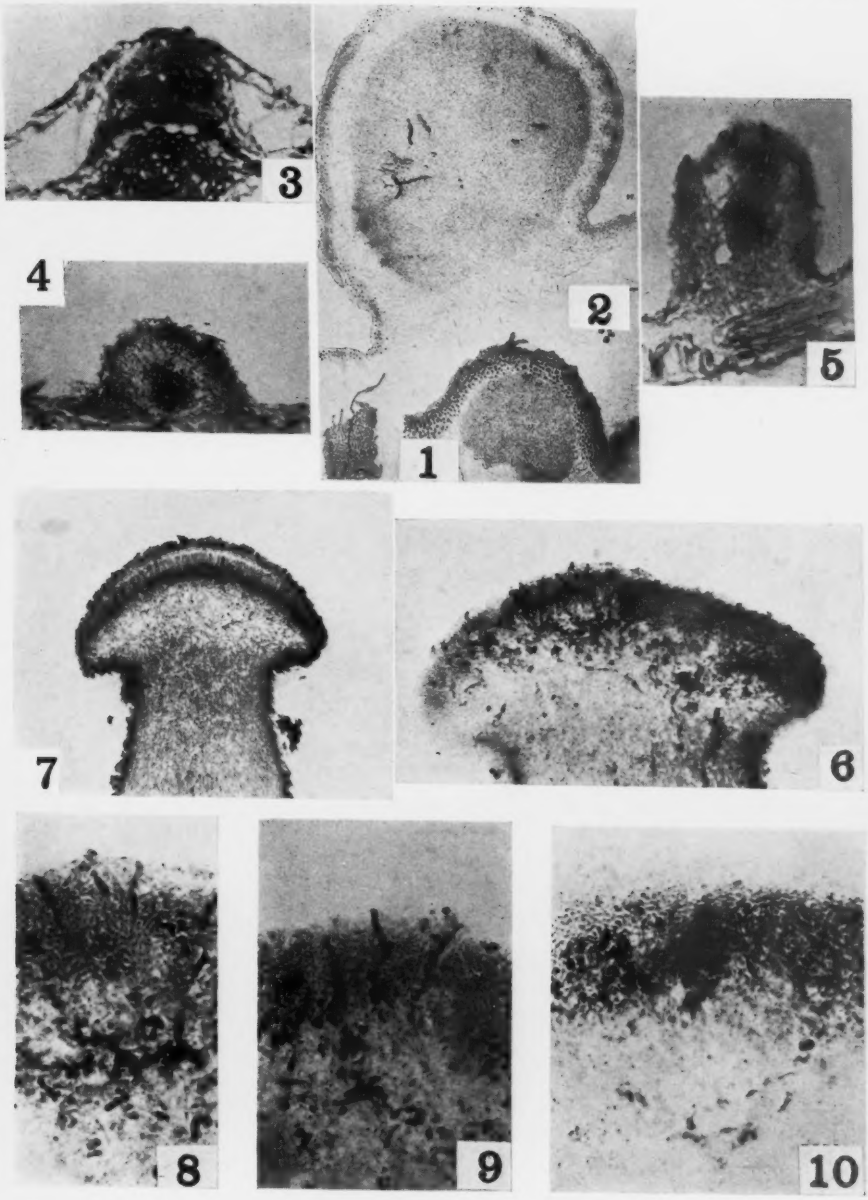
7. It is suggested that the progress of evolution in these plants has been from a type in which fertilization took place through the agency of the trichogyne, and has been marked by gradual reduction of the sex organs.

This investigation was conducted under the direction of Professor J. H. FAULL of the University of Toronto, to whom the writer would acknowledge his great indebtedness for assistance and criticism through the whole course of the work, and also for liberal contributions of material.

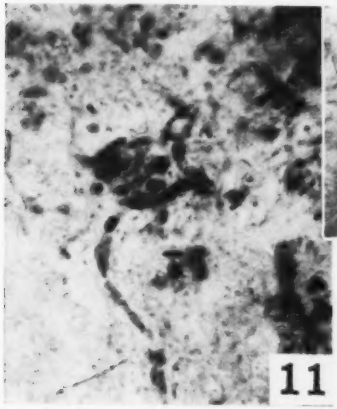
UNIVERSITY OF TORONTO

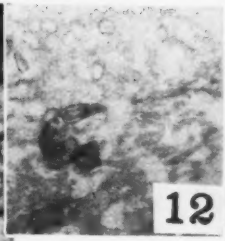
LITERATURE CITED

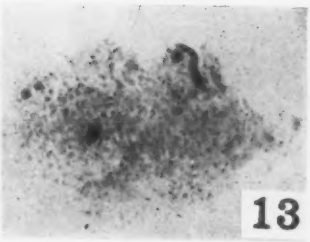
1. BLACKMAN, V. H., and WELSFORD, E. J., The development of perithecium of *Polystigma rubrum* DC. Ann. Botany **26**:761–767. 1912.
2. BROOKS, F. T., The development of *Gnomonia erythrostoma* Pers. Ann. Botany **24**:585–606. 1910.
3. BROWN, H. B., Studies in the development of *Xylaria*. Ann. Myc. **11**:1–13. 1913.
4. BROWN, W. H., The development of the ascocarp of *Leotia*. BOT. GAZ. **50**:443–459. 1910.
5. ———, The development of the ascocarp of *Lachnea scutellata*. *Ibid.* **52**:273–305. 1911.
6. DAWSON, MARIA, On the biology of *Poronia punctata*. Ann. Botany **14**:245–260. 1900.
7. DITTRICH, GUSTAV, Zur Entwickelungsgeschichte der Helvellineen. Cohn's Beiträge **8**:17–52. 1898.
8. DODGE, B. O., Artificial cultures of *Ascobolus* and *Aleuria*. Mycologia **42**:218–222. 1912.
9. ———, The morphological relationships of the Florideae and the Ascomycetes. Bull. Torr. Bot. Club **41**:157–202. 1914.
10. DUFF, G. H., Development of the Geoglossaceae. BOT. GAZ. **69**:341–346. 1920.
11. DURAND, E. J., The Geoglossaceae of North America. Ann. Myc. **6**: 388–477. 1908.
12. FINK, BRUCE, The nature and classification of lichens. I. Views and arguments of botanists concerning classification. Mycologia **3**:231–269. 1911.
13. FISCH, C., Beiträge zur Entwickelungsgeschichte einiger Ascomyceten. Bot. Zeit. **40**:865–870, 875–882. 1882.
14. FITZPATRICK, H. M., The development of the ascocarp of *Rhizina undulata* Fr. BOT. GAZ. **63**:282–296. 1917.
15. ———, Sexuality in *Rhizina undulata* Fries. *Ibid.* **63**:201–226. 1918.
16. KRABBE, G., Entwickelung, Sprossung, und Theilung einiger Flechtenapothecien. Bot. Zeit. **40**:65–83, 89–99, 105–116, 121–142. 1882.
17. MCCUBBIN, W. A., Development of the Helvellineae. I. *Helvella elastica*. BOT. GAZ. **49**:195–206. 1910.
18. NIENBURG, W., Beiträge zur Entwickelungsgeschichte einiger Flechtenapothecien. Flora **98**:1–40. 1908.
19. ———, Zur Entwickelungsgeschichte von *Polystigma rubrum*. Zeit. Bot. **6**:369–400. 1914.
20. SCHROETER, J., In Engler und Prantl. Die natürlichen Pflanzenfamilien. 1894.
21. VAUGHAN, R. E., A method for the differential staining of fungus and host cells. Ann. Mo. Bot. Gard. **1**:1914.

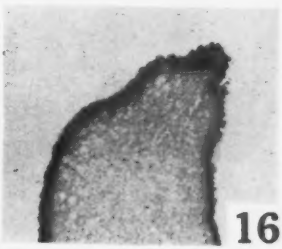


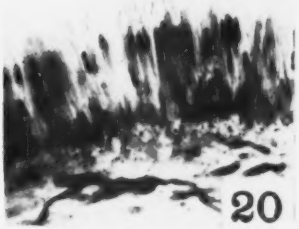
DUFF on GEOGLOSSACEAE

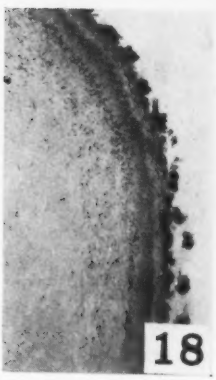


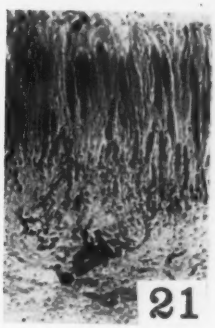


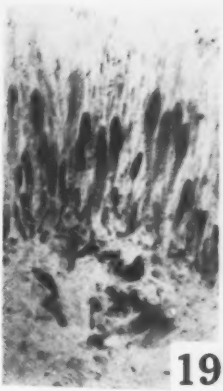




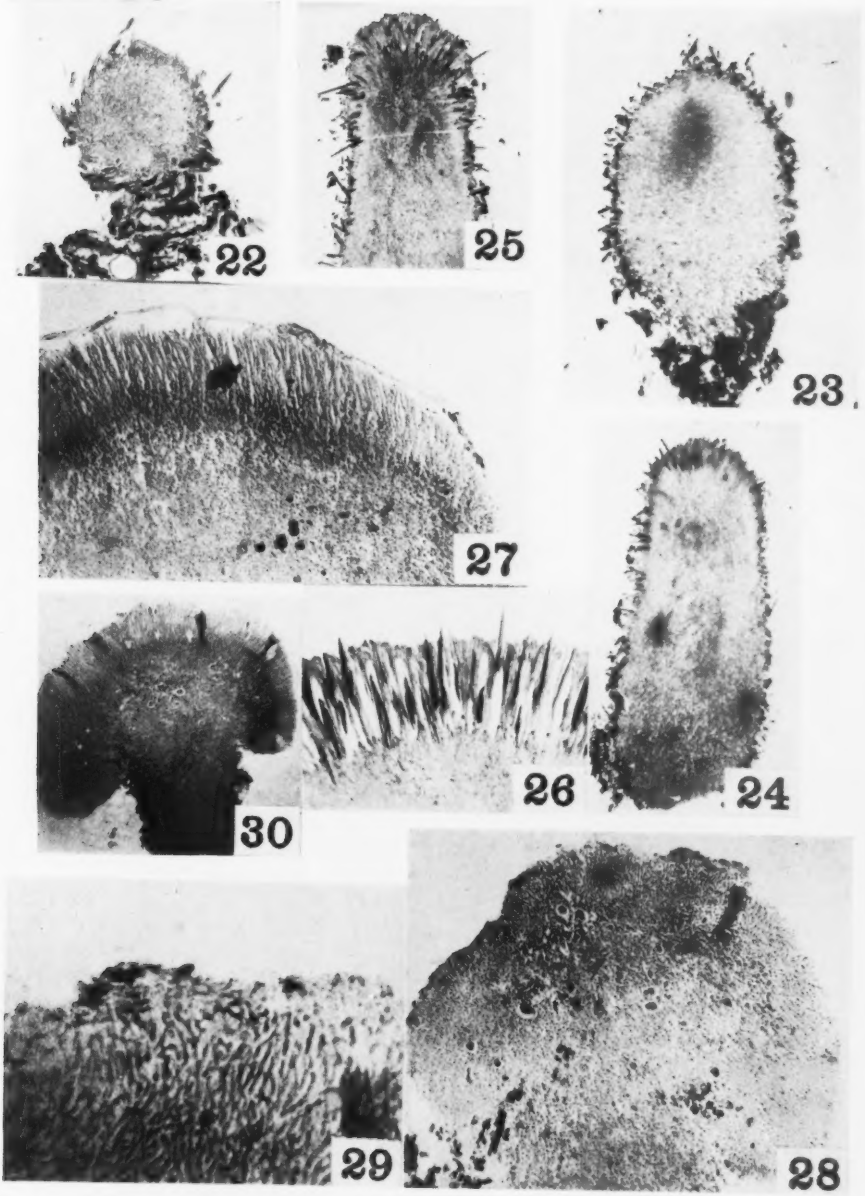




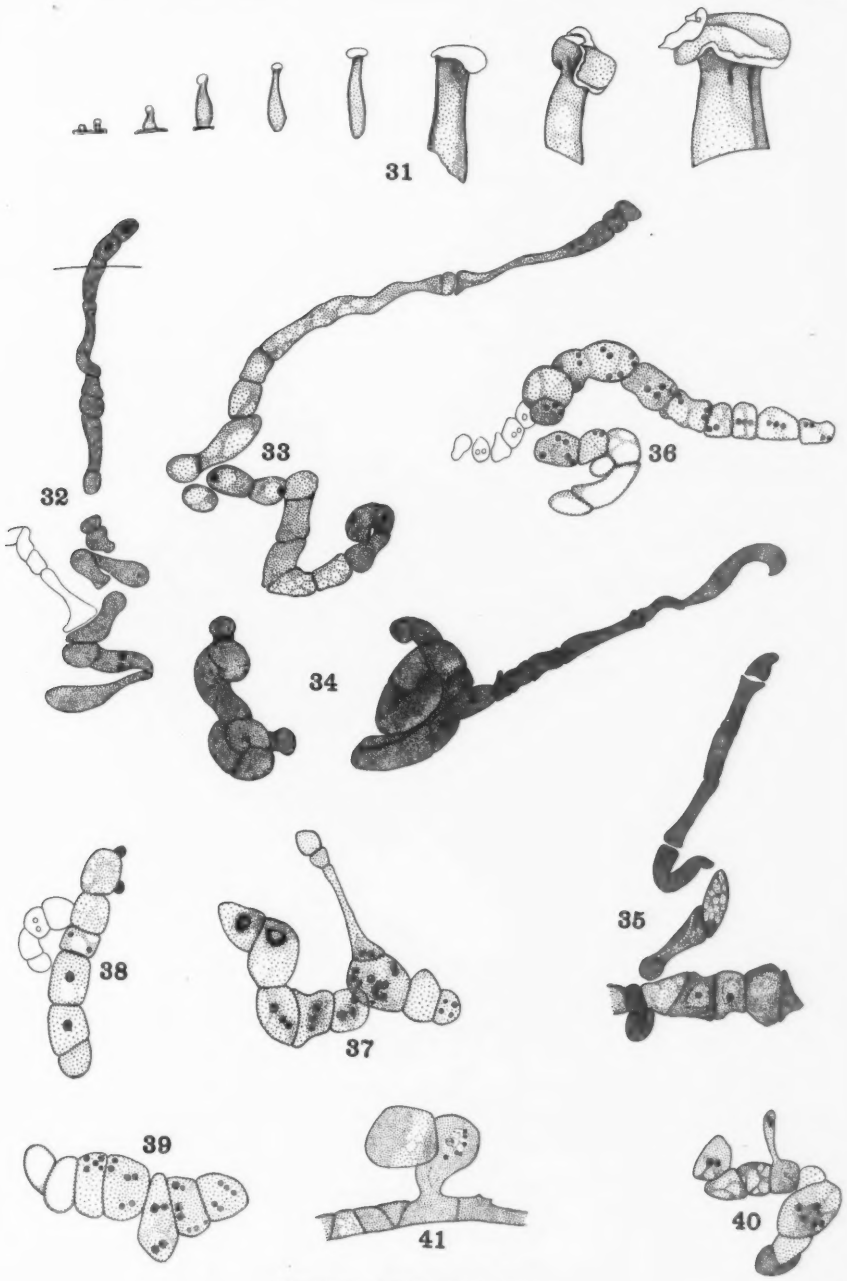




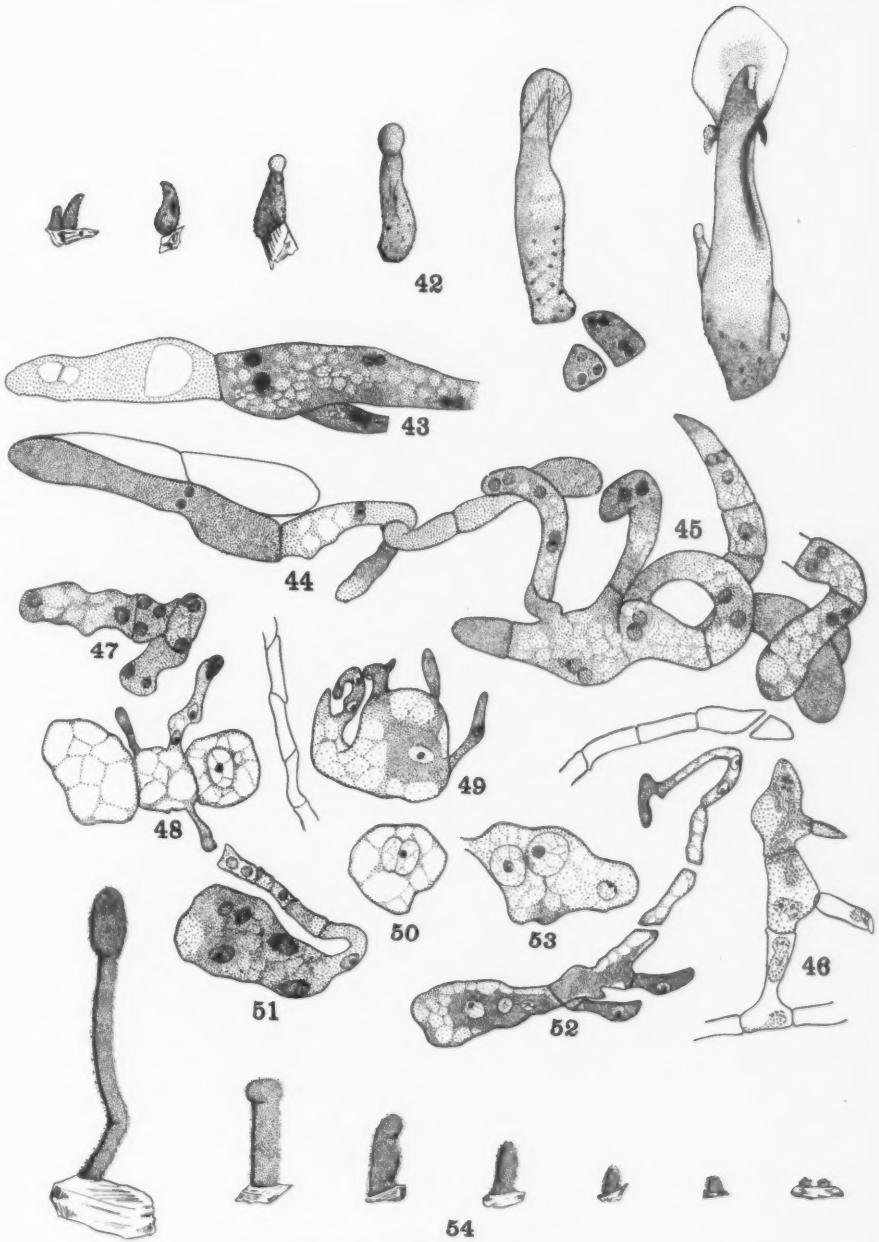
DUFF on GEOGLOSSACEAE



DUFF on GEOGLOSSACEAE



DUFF on GEOGLOSSACEAE



DUFF on GEOGLOSSACEAE

EXPLANATION OF PLATES VIII-XII

FIGS. 1, 2.—*Icmadophila aeruginosa:* sections of young ascocarps, from NIENBURG; fig. 1, ×140; fig. 2, ×100.

FIGS. 3–5, *Cudonia lutea:* sections of young fructifications, showing generative hyphae and origin of veil; fig. 3, ×140; fig. 4, ×160; fig. 5, ×140.

FIG. 6.—*C. lutea:* section of cap of ascocarp, showing procarps and trichogynes; ×130.

FIG. 7.—*C. lutea:* section of ascocarp, showing veil inclosing young hymenium; ×70.

FIGS. 8, 9.—*C. lutea:* procarps and trichogynes enlarged; ×390.

FIG. 10.—*C. lutea:* ascogenous hyphae passing from procarp to hymenium; ×370.

FIG. 11.—*C. lutea:* lower portion of procarp with generative hyphae; ×530.

FIG. 12.—*C. lutea:* old procarp with empty cells; ×430.

FIG. 13.—*C. lutea:* first section of series through cap, showing projecting trichogyne; ×500.

FIGS. 14–17.—*Spathularia velutipes:* sections of young ascocarps; fig. 14, ×50; fig. 15, ×35; fig. 16, ×35; fig. 17, ×40.

FIG. 18.—*S. velutipes:* sections of cap, showing veil inclosing young hymenium; ×150.

FIGS. 19–21.—*S. velutipes:* procarps and ascogenous hyphae; fig. 19, ×400; fig. 20, ×250; fig. 21, ×310.

FIGS. 22–25.—*Trichoglossum hirsutum:* sections of young ascocarps; fig. 22, ×100; fig. 23, ×80; fig. 24, ×33; fig. 25, ×27.

FIG. 26.—*T. hirsutum:* hymenium; ×85.

FIGS. 27, 28.—*Leotia lubrica:* sections of young ascocarps, showing overlying fragments of tissue; fig. 27, ×140; fig. 28, ×175.

FIG. 29.—*L. lubrica:* fragment of overlying tissue enlarged; ×695.

FIG. 30.—*L. lubrica:* section of ascocarp, showing enlarged cells resembling procarps of *Spathularia;* ×70.

FIG. 31.—*Cudonia lutea:* series of ascocarps at different stages; ×3.

FIGS. 32–35.—*C. lutea:* procarps and trichogynes; ×1125.

FIGS. 36–40.—*C. lutea:* procarp cells at multinucleate stage; ×1125.

FIG. 41.—*C. lutea:* two cells of procarps with degenerated contents, showing also generative hypha; ×1125.

FIG. 42.—*Spathularia velutipes:* series of ascocarps at different stages; ×3.

FIGS. 43–45.—*S. velutipes:* procarp cells; ×2000.

FIG. 46.—*S. velutipes:* old procarp cells with depleted contents; ×1125.

FIGS. 47–53.—*Leotia lubrica:* large cells of cap, showing nucleation and in some cases ascogenous hyphae; ×1125.

FIG. 54.—*Trichoglossum hirsutum:* series of ascocarps at different stages; ×3.

A MORPHOLOGICAL STUDY OF THE UMBELLIFERAE

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 298

HILARY STANISLAUS JURICA

(WITH PLATES XIII, XIV)

Introduction

The fact that the Umbelliferae are so extensive and so well distributed throughout the northern university zone has made them an object of frequent study. CÉSALPIN (**9**) was the first to assemble the different members of the Umbelliferae into a separate group, not only on the basis of their umbellate inflorescence, but also on the basis of a secondary character, the two-celled ovary, each cell of which gives rise to a single seed. MORISON (**51**) recognized the family on the basis of the same characters as CÉSALPIN, but added a number of other plants, especially some of the Valerianaceae and *Thalictrum*, which of course, were not destined to remain within this family. To these, he applied the name of imperfect Umbelliferae. According to GÉNEAU DE LAMARLIÈRE (**27**), however, it was left for HERMANN (1690) to establish a rational division for this family, namely, (1) species with ovate seeds; (2) species with hairy or spiny fruits; and (3) species with large and flattened fruits. Following HERMANN, MAGNOL (**27**), in 1709, divided the family into four groups based on the character of the surface of the fruit: (1) fruit ribbed, (2) fruit large, (3) fruit spiny, and (4) fruit long.

The classification of the Umbelliferae entered upon a new phase with LINNÉ (**45**), who selected or rather borrowed from his contemporary ARTHEDIUS the row of bracts of the involucre and of the involucel as a principal character, upon which he based his division of the Umbelliferae, with the already mentioned external features of the fruit as secondary characters. At this time the essential distinction between a cyme and an umbel was not considered, and accordingly very many forms were included, which later workers, notably ADAMSON (**1**), CRANTZ (**15**), SPRENGEL (**63**), HOFFMANN

(32, 33), KOCH (42), DE CANDOLLE (17), etc., assigned to other families. A somewhat extensive discussion of the history of the classification of Umbelliferae may be found in the work of GÉNEAU DE LAMARLIÈRE (27). For American workers, COULTER and ROSE (13) listed a condensed bibliography of all the works containing new names or new descriptions of Umbelliferae found within the North American range, from LINNAEUS' *Species Plantarum* (1753) to that of CONGDON (1900).

The first morphological work of note upon the Umbelliferae was that of TITTMANN (65), whose figures showed the germination of some species with great exactness. Influenced by the research work of their age, or at times by the somewhat peculiar nature of the plant at hand, a further study of the germination of a large number of genera and species was made by DE CANDOLLE (17), TREVIRANUS (69, 71), BERNHARDI (4), KIRSCHLEGER (40), IRMISCH (36, 37), VAN TIEGHEM (76), GÉNEAU DE LAMARLIÈRE (24, 25, 26, 27), DOMIN (18), DRUDE (20), HOLM (35), and MÖBIUS (49).

Although DE CANDOLLE (16) had already described the stem of *Ferula*, whose medullary bundles could easily lead one to mistake it for a monocotyledon stem, it was left for HOFFMANN (34) to present us with the first extensive anatomical work upon the Umbelliferae. His study of the roots of the plants of this family contains many interesting details, but it is to be regretted that he failed to distinguish the root from the rhizome, and at times even confounded it with the lower part of the aerial stem. Moreover, he paid no attention to order and very little to development.

Further anatomical studies were made by JOCHMANN (38), REICHARDT (58), DUCHARTRE (21), BEHUNECK (3) FAURE (22), GERARD (28), TRECUL (68), COURCHET (14), HOLM (35), KLAUSCH (41), GÉNEAU DE LAMARLIÈRE (27), NOENEN (55), and NESTEL (54). The work of MÖBIUS (49), however, deserves special attention, for the parallel-veined leaves of numbers of species of *Eryngium*, together with the general aspect of their gross morphology, lead many taxonomists to suspect an analogy in their anatomy to that of some of the monocotyledons. Accordingly, we have species like *Eryngium yuccifolium* Michx., *E. bromeliaefolium* Delar., *E. pandanifolium* Chan., *E. luzulaefolium* Chan., *E. junceum* Chan.,

and *E. scirpinium* Chan. In his extensive work covering the anatomy of the leaf, stem, rhizome, and root, MÖBIUS showed that this similarity is only apparent, and that in reality the stem of *Eryngium* is not merely a dicotyledon, but is one of an advanced type.

A rather unique and quite extensive study of the mechanical tissue in the stem and leaf was made by FUNK (**23**), as recently as 1912.

The formation of the leaves, umbels, and gross morphology next received attention from workers like JOCHMANN (**38**), BUCHENAU (**7**), MOHL (**50**), ROSSMANN (**60**), CLOS (**10, 11**), GERARD (**28**), KLAUSCH (**41**), DOMIN (**18, 19**), PETERSEN (**57**), RENNERT (**59**), TERNETZ (**66**), WRETSCHKO (**84**), and GRIESEBACH (**29**).

The oil ducts or secreting canals were studied by MEYEN (**47**), JOCHMANN (**38**), MEYER (**48**), VAN TIEGHEM (**73, 74, 75**), MÜLLER (**53**), MOYNIER DE VILLEPOIX (**52**), LANGE (**43**), and VUILLEMIN (**79**).

PAYER (**56**), TRECUL (**67**), and JOCHMANN (**38**), whose works appeared but a few months apart, were the first to attempt the organogeny of this family. In the formation and development of the leaves all three disagree, and all differ from the account given by GRIESEBACH (**29**), but the accounts of PAYER (**56**) and JOCHMANN (**38**) agree in regard to the floral development and also with the accounts of SIELER (**62**) and HANNAH (**31**). SIELER, however, interprets the "calycis primordium" of these workers as a special kind of organ, which gives rise to the calyx, and naturally he finds fault with the seemingly existing "primordialkelch," a view which I failed to receive from the reading of both PAYER'S and JOCHMANN'S works. JOCHMANN'S work especially evinces great care, and no doubt, had modern technique been available in his day, the embryogeny would have been included. Like all the earlier workers, JOCHMANN begins his study with germination, but, unlike them, he pays special attention to the root, rhizome, and stem, their anatomy, and their oil ducts. He then proceeds to discuss the development of the leaves, umbels, flower, stylopodium, style, "gynoecium," pericarp, and the seed.

Among other workers dealing with the development or histology of the fruit may be mentioned LANESSAN (**44**), BARTSCH (**2**),

TREVIRANUS (70), and TANFANI (64). Quite recently an approach was made to the study of embryogeny by CAMMERLOHER (8), who studied the "Samenanlagen" of a large number of genera; and MARTEL (46) presented us with an anatomy of the flower.

Summing up all the work done, however, we find that the classification, gross morphology, anatomy, mechanical tissue, oil ducts, development of leaves and inflorescences, floral development, and development of fruit in this family are well covered, but embryogeny proper, endosperm formation, and the embryo still remain to be studied. It is with a view to filling this gap that this investigation has been entered upon.

Methods

The material used in this study was collected at different intervals in the vicinity of Lisle, Illinois. The fresh material was killed in a stock solution of chromo-acetic acid, imbedded in paraffin, and stained usually with either safranin and Delafield's haemotoxylin, or safranin, gentian violet, and orange G (omitted in a few cases).

The study centers about *Eryngium yuccifolium*, with frequent comparisons with other genera, especially *Sium cicutaefolium*.

Floral development

No attempt has been made to present a study of the development of the inflorescence or umbel, for that has already been well studied by workers listed in the introduction. Nevertheless, since the umbels of *Eryngium yuccifolium* consists of distinct compact heads, a short note in regard to the head will not be out of place.

The central or apical head of each umbel develops earlier and more rapidly than the encircling members, hence by the time the central head becomes visible to the naked eye, longitudinal sections show that the encircling heads develop in the axils of the bracts subtending the umbel, in much the same manner as described by JURICA (39) for the heads of *Dipsacus sylvestris* (fig. 1).

As already has been described by JOCHMANN (38) for *Eryngium planum*, all the flowers in the heads of *Eryngium yuccifolium* arise from the axils of bracts spirally arranged. The blossoming begins

at the base and extends toward the apex (fig. 1). The individual epigynous flowers appear as an undifferentiated mass of cells, somewhat rounded at first (fig. 2), but soon broaden out, so that the individual sepal primordia forming the calyx (which is well pronounced in *Eryngium yuccifolium*) are distinctly visible (figs. 3, 4). This is soon followed by a perfect and regular acropetal succession, presenting the sequence sepals, petals, stamens, and carpels, in perfect accord with the account given by HANNAH (**31**) for *Sanicula marilandica;* by PAYER (**56**) for *Heracleum Sphondylium* (and other species of *Heracleum*), *Carum*, *Aegopodium*, *Anethum*, *Phellandrium aquaticum*, etc.; and by SIELER (**62**) for *Heracleum*, *Sphondylium*, *Chaerophyllum bulbosum*, *Cicuta virosa*, *Daucus Carota*, *Peucedanum cervaria* Lat., *Angelica sylvestris*, etc. (figs. 2–7). It is well to note, however, that SEILER (**62**) is more concerned with the study of the sequence of the appearance of individual members within a cycle than with the sequence of the cycles themselves.

JOCHMANN (**38**) had noted that, although the calyx primordia make their regular appearance in *Aegopodium*, etc., they fail to continue in their development ("in pristino statu remanent, et quo, magis flos accrescit, eo magis evanescunt"), and thus apparently simply remain as calyx teeth, and in many genera even these are obsolete and hardly distinguishable (figs. 9, 10).

Ovules

The carpels are distinctly two in number at first, but soon unite along their inner face, so that in cross-section they appear to be semicircular (fig. 7); or as PAYER (**56**), JOCHMANN (**38**), SEILER (**62**), and CAMMERLOHER (**8**) would have it in the forms studied by them, "semilunar" in shape. In each of the four free ends of the coalesced carpels an anatropous ovule begins to develop (figs. 11, 12), one of which soon stops, however, while the other continues in its normal development. This results in a hanging anatropous ovule, with the raphe turned inward and the micropyle outward (fig. 14). This seems to be quite general for the entire family, for it has been found in all the species studied, and it is in accord with the account of CAMMERLOHER (**8**), who studied the "Samenanlagen" in thirty-seven genera and forty-five species. Moreover, JOCHMANN

(38), PAYER (56), and SEILER (62) have noted it in their work. Nevertheless, it is not out of place to note that, in one exceptional case, all the flowers of one head of *Eryngium yuccifolium* developed two normal ovules in each ovary cavity (fig. 13).

Megaspore and embryo sac

By the time the ovule has reached the stage shown in fig. 17, the nucellus has become quite prominent, and the hypodermal archesporium is easily recognizable (fig. 18). This figure shows the megaspore mother cell nucleus in synapsis, which indicates that the reduction division is about to occur. The successive stages in the development of the megaspore, resulting in a linear row of four megaspores (fig. 19), as well as the destruction of the potential megaspores, present no essential deviations from the process as ordinarily described, for the embryo sac develops from the innermost megaspore, and *Sium cicutaefolium* is no exception in this case (figs. 15, 16). The nucellus has only a single layer of cells surrounding the megaspore, but the absence of tapetal cells is well compensated by the presence of a nutritive apparatus (figs. 16, 20–25) in the chalazal region. The nucellus undergoes but slight development, and then begins to break down (figs. 16, 21–25). Even in the development of the embryo sac there is nothing unusual. The megaspore nucleus divides by three successive divisions, and at first an eight-nucleate, and then a seven-nucleate embryo sac is the result. The amount of protoplasm in the developing sac is comparatively small, frequently resulting in the presence of very large vacuoles, not only in the embryo sac proper, but in the synergids and oosphere as well (figs. 23–25). The antipodal cells, three in number, can easily be seen in the embryo sac before the polar nuclei fuse, and generally are arranged in the form of a triangle (fig. 24). They soon break down, however, and only rarely can be distinguished at a later stage (fig. 16), that is, they are somewhat ephemeral, breaking down shortly before or after the fusion of the polar nuclei.

Endosperm and embryo

Shortly after double fertilization the endosperm nucleus begins to divide, forming an endosperm consisting of free nuclei (figs. 26,

27). The early divisions of the endosperm nuclei occur more rapidly than those of the embryo, and very soon cell walls make their appearance. Even after the formation of cell walls, the endosperm continues to form so rapidly that when the seed is mature the small, insignificant, yet massive embryo (figs. 28–30) is practically inclosed in rich endosperm tissue. In its development the embryo does not always divide into regular octants (figs. 28–30), but at times is quite irregular. Very frequently in its early development it looks more like a pteridophyte embryo (fig. 28), and is characterized by a long suspensor.

Relationships

The Umbelliferae are very closely related to the Araliaceae and Cornaceae, with which families they form an alliance or order known as the Umbellales, or the "Umbelliflorae" of ENGLER. Although several workers, notably HALLIER (**30**), WETTSTEIN (**83**), and WERNHAM (**82**), have elaborated various tables showing the probable phylogenetic relationships, the scheme of ENGLER is still followed by most workers. In ENGLER'S classification the dicotyledons are divided into two great divisions, the Archichlamydeae and Sympetalae, which, according to COULTER and CHAMBERLAIN (**12**), however, show no sharp distinction, for "sympetalous forms occur among the former, and polypetalous forms among the latter." The distinction is laid principally upon a single character, namely, apetaly or polypetaly for the Archichlamydeae, and sympetaly for the Sympetalae. Without doubt this is pressing a single character too far, and as a result the Umbellales "stand so stiffly apart from other Archichlamydeae as to raise the question whether they do not really belong among the higher Sympetalae" (**12**).

It will not be out of place to indicate such evidence as there is for such an assumption from the taxonomists' point of view. For this purpose the recent extensive work of VIGUIER (**78**) may be cited. In his chapter dealing with the relationship of the Araliaceae with other families, he definitely proves the close relationship of the three families forming the order Umbellales, claiming, for example, that the only character which separates the Araliaceae from the Umbelliferae is the drupaceous fruit of the former, whereas the two

families have a number of general characters in common. He also states that not a single anatomical character permits an absolute separation of the two families. After describing the details of this anatomical relationship, he concludes that the Umbelliferae, with the Araliaceae, form a continued morphological series and a very natural group. VIGUIER next shows that the Cornaceae are closely related to the Araliaceae, claiming that even good systematists at times take a single genus out of one of the families and put it into another. WANGERIN (80) and WARMING (81), however, would separate the Cornaceae from the Umbellales on account of the nature of the ovule. No less apparently conclusive is the account of WETTSTEIN (83) in regard to the inter-relationship of the three families forming the Umbellales.

With this inter-relationship established, we can safely return to VIGUIER (78), who compares the Pittosporaceae with the Umbelliferae and Araliaceae on the basis of the presence of secreting canals, for which reason VAN TIEGHEM (77) has added this as a fourth family of the order Umbellales, and his studies upon the ovule of the Pittosporaceae confirm his view.

This interpretation seems plausible, for the ovary of the Pittosporaceae is likewise bicarpellate, although the ovules themselves are free and generally parietally arranged in two rows. The single integument, and the fact, as WETTSTEIN (83) has it, that the corolla is very often somewhat sympetalous ("Korolle manchmal etwas sympetal"), prove that at any rate the Pittosporaceae are out of place among the Rosales, and no doubt belong among the Sympetalae. The inclusion of the Pittosporaceae in the Umbellales by VAN TIEGHEM likewise demands the transfer of the entire Umbellales to the sympetalous dicotyledons. Accordingly they are not to be viewed as an order closing up the Archichlamydeae line, the next of kin to the Myrtales. This is in spite of the fact that the Umbellales have some characters in common with some of the Myrtales, especially with *Hippurus* of the Haloragidaceae, such as epigyny, single integument, etc., for they stand too sharply apart from the rest. The position of families forming the order Myrtales certainly deserves a reconsideration, and this has already been done in part by SCHINDLER (61), who

separated *Hippurus* from the Haloragidaceae on the basis of its morphological characters, and made a new family entitled Hippuridaceae. Further rearrangement no doubt will follow later, when more morphological work has been done on the group.

Viewing the Umbelliferae from a morphological standpoint, it is clear that separate petals are the only character which they have in common with the Archichlamydeae, and they even lack sympetaly, if the Umbellales are taken as a whole on the basis of VAN TIEGHEM'S work on the Pittosporaceae; for, according to BESSEY (5), "one organ may be advancing while another is retrograding."

"The complete cyclic arrangement of floral members associated with definite numbers" (12), the single integument (fig. 17), the anatropous ovule (fig. 14), the absence of parietal tissue of the megasporangium (fig. 18), the small nucellus (fig. 18), and the complete tetrad of the megaspores (fig. 19) of the Umbelliferae, all of which are general characters of Sympetalae in contrast with those of the Archichlamydeae, from which the Umbelliferae stand so stiffly apart, prove that the Umbellales in reality belong among the Sympetalae.

The question now arises, if they are to be placed among the Sympetalae, what is their relative position? This is not difficult to answer, for the epigynous nature of their flower places them surely above the Tubiflorales, and their other floral characters put them below the Campanulales. Hence, a position in the neighborhood of the Rubiales is without question. C. E. BESSEY (5), E. A. BESSEY (6), and WETTSTEIN (84) consider the Umbellales as giving rise to the Rubiales. WERNHAM (82) notes that "within both cohorts," as he calls them, "the progress from polycarpellary to a bicarpellary gynoecium is observable; in both the ovary is only very rarely unilocular; and in both the androecium is primitively isomerous with the corolla, and the latter primitively regular." Accordingly, he recognizes the "Umbelliflorae as the representatives of a side branch from the calycifloral (rosalium) plexus, and the Rubiales as another such side branch of this stock." All this is to fit in with his scheme of a polyphyletic origin of the Sympetalae.

On the other hand, HALLIER (30) would have a common "Umbellifloren" stock reaching from the Terebinthaceae, and giving rise to Cornaceae, and through these to two branches, namely, to Umbelliferae on one side and to Rubiales on the other. From this it is evident that, although these systematists disagree as to details, they agree upon the fact that the Umbellales are related to Rubiales. Recognizing ENGLER'S scheme of classification as the one more commonly and more widely accepted, and in view of the numerous facts, both from the taxonomic and morphological field, I suggest that the Umbellales be placed among the Sympetalae parallel with the Rubiales. Even if the viewpoint of WANGERIN (80) and WARMING (81) in regard to the Cornaceae should be proved to be correct and universally accepted, this would not affect the remaining families of the Umbellales, for then the Cornaceae would be transferred to a position in close association with the Caprifoliaceae.

Summary

1. The floral development shows an acropetal succession of floral cycles, namely, sepals, petals, stamens, and carpels.

2. In the genera in which the sepals are represented by mere calyx teeth or are obsolete, the calyx primordia also make their appearance, but fail to develop any further.

3. The carpels are two in number and later fuse to form the ovary.

4. Two anatropous ovules begin to develop in each cavity, but usually the lower one reaches maturity.

5. The hanging anatropous ovule has a single integument.

6. The nucellus is very small, and the hypodermal archesporial cell is easily recognizable.

7. The megaspore mother cell produces a perfect linear tetrad, as a result of two successive divisions.

8. The embryo sac develops from the innermost megaspore, the three others aborting.

9. A regular eight-nucleate and subsequent seven-nucleate embryo sac results from three successive divisions of the megaspore, followed by the fusion of the polar nuclei.

10. The antipodals are somewhat ephemeral, breaking down either shortly before or after the fusion of the polar nuclei.

11. The endosperm nucleus is the first to divide after double fertilization, and for a while continues to produce free nuclei, but cell walls soon appear.

12. The fertilized egg is slow to divide, and undergoes no extensive development, so that the ripe seed has a small embryo imbedded in rich endosperm tissue.

13. The suspensor is somewhat long.

14. The morphological features of the Umbelliferae show that they are out of place among the Archichlamydeae, and that they belong among the Sympetalae, in spite of their separate petals, just as *Agapanthus* is a good monocotyledon in spite of its two cotyledons.

15. The epigynous nature of the flower in close affinity with that of the Rubiales warrants the placing of the Umbellales about parallel with the Rubiales, among the Sympetalae, that is, as a side line having a common origin with the Rubiales.

Acknowledgment is due to Dr. CHARLES J. CHAMBERLAIN for proposing the problem, as also for suggestions during the course of the investigation.

ST. PROCOPIUS COLLEGE
LISLE, ILL.

LITERATURE CITED

1. ADAMSON, MICHAEL, Familles des plantes. Paris. 1763.
2. BARTSCH, E., Beiträge zur Anatomie und Entwicklung der Umbelliferenfrüchte. Diss. Breslau. 1882.
3. BEHUNECK, H., Zur Anatomie von *Oenanthe crocata*. Diss. Kiel. 1879.
4. BERNHARDI, J. J., Über die merkwürdigsten Verschiedenheiten des entwickelten Pflanzenembryo und ihren Werth für die Systematik. Linnaea **7**:561–613. 1832.
5. BESSEY, C. E., The phylogenetic taxonomy of flowering plants. Ann. Mo. Bot. Gard. **2**:109–164. 1915.
6. BESSEY, E. A., The origin of the Anthophyta. Ann. Rep. Mich. Acad. Sci. **17**:142–152. 1915.
7. BUCHENAU, F., Der Bluenthenstand und die Zweigbildung bei *Hydrocotyle vulgaris*. Bot. Zeit. **18**:365. 1860.

8. CAMMERLOHER, H., Studien über die Samenanlagen der Umbelliferen und Araliaceen. Oest. Bot. Zeitsch. **60**:289–300, 356–360. 1910.
9. CÉSALPIN, ANDREA, Libri XVI de plantis. Florence. 1583.
10. CLOS, D., La feuille et la ramification dans les Ombellifères. Toulouse Acad. Sci. Mem. **6**:195–231. 1874.
11. ———, De quelques particularités de l'inflorescence afférentes au *Bupleurum fruticosum*, etc. Assoc. Franc. C.R. **2**:460–464. 1890.
12. COULTER, J. M., and CHAMBERLAIN, CHAS. J., Morphology of angiosperms. New York. 1903.
13. COULTER, J. M., and ROSE, J. N., Monograph of the North American Umbelliferae. Washington. 1902.
14. COURCHET, L., Étude anatomique sur les Ombellifères et sur les principales anomalies de structure que présentent leurs organes végetatifs. Ann. Nat. Bot. VI. **17**:107–129. 1884.
15. CRANTZ, H. J. N., Classis Umbelliferarum emendata. Lipsiae. 1767.
16. DE CANDOLLE, A. P., Organographie végétale. **1**:164. 1827.
17. ———, Mémoire sur la famille des Ombellifères. Paris. 1829.
18. DOMIN, KAREL, Morfologická a fylogenetická studia o čeledi Umbellifer Část I. Rozpravy České Akad. Čis. Fr. Jos. Třida II. **20**:1–20. 1908.
19. ———, Morfologická a fylogenetická studia o čeledi Umbellifer. Část II Rozpravy Ceské Akad. Cis. Fr. Jos. Třida II. **3**:43–98. 1909.
20. DRUDE, OSCAR, Über die systematische Anordnung der Umbelliferen. Deutsch. Natf. Verh. **2**:164–165. 1896.
21. DUCHARTRE, P., Note sur un cas de formation de racines adventives intérieures. Bull. Soc. Bot. Fr. **16**:26–29. 1869; Note sur une particularité observée dans *Oenanthe crocata* L. *Ibid.* **16**:363. 1869.
22. FAURE, JOS., L'accroissement et la marche des faisceaux dans les petioles de l'*Archangelica*. Bull. Soc. Bot. Lyon. **9**:310–311. 1882.
23. FUNK, G., Beiträge zur Kenntniss der mechanischen Gewebesysteme in Stengel und Blatt der Umbelliferen. Bot. Centralb. Beih. **29**:219–295; 1912.
24. GÉNAU DE LAMARLIÈRE, L., Sur la germination de quelques Ombellifères. Assoc. Franc. C.R. **2**:480–484. 1891; and Rev. Gen. Bot. **5**:159–171. 224–229; 258–264. 1893.
25. ———, Structure comparée des racines renflées de certaines Ombellifères. Paris Acad. Sci. C.R. **112**:1020–1022. 1892.
26. ———, Sur la germination et le développement du tubercule du *Conopodium denudatum* Koch. Ass. Franc. C.R. **2**:445–449. 1892.
27. ———, Mémoire: Recherches morphologiques sur la famille des Ombellifères. Lille. 1893.
28. GERARD, R., Structures de l'axe des *Oenanthe* et considérations sur les formations anomales. Bull. Soc. Bot. **30**:299–304. 1883.
29. GRIESEBACH, AUG. H. R., Nachtrag zu den Beobachtungen über das Wachsthum der Blätter. Wiegmann's Archiv X. **1**:345–347. 1844.

30. HALLIER, H., L'origine et le système phylétique des Angiosperms, exposés à l'aide de leur arbre généalogique. Arch. Neerland. Sci. III. **1**:146. 1912.
31. HANNAH, M., Comparative study of epigyny in certain monocotyledons and dicotyledons. Trans. Amer. Micr. Soc. **35**:207–220. 1916.
32. HOFFMANN, G. F., Genera plantarum Umbelliferarum. Mosquae. 1816.
33. ———, Syllabus plantarum Umbelliferarum. Mosquae. 1814.
34. HOFFMANN, HERMANN, Über der Wurzeln der Doldengewächse. Flora **32**:16–25; 721–728. 1848; **33**:385–389; 401–405; 657–665. 1849; **34**:513–519; 529–535. 1850; **35**:225–233; 241–256. 1851.
35. HOLM, H., *Erigenia bulbosa* Nutt., a morphological and anatomical study. Amer. Jour. Sci. **11**:63–72. 1901.
36. IRMISCH, T., *Carum bulbocastanum* und *Chaerophyllum bulbosum* nach ihrer Keimung. Abhandl. Naturf. Gesellsch. Halle **2**:47. 1854.
37. ———, Über Keimung von *Bunium creticum*. Flora **16**:33–42. 1858.
38. JOCHMANN, E. C. G. G., De Umbelliferarum structura et evolutione nonnulla. Diss. Vratislaviae. 1854.
39. JURICA, H. S., Development of the head and flower of *Dipsacus sylvestris*. BOT. GAZ. **71**:138–145. 1921.
40. KIRSCHLEGER, F., Über das Keimen des *Chaerophyllum bulbosum* L. Flora **28**:401–402. 1845.
41. KLAUSCH, P., Über die Morphologie und Anatomie der Blätter von *Bupleurum*, etc. Diss. Leipzig. 1887.
42. KOCH, G. D. J., Generum tribumque Umbelliferarum nova dispositio. Nova Acta Acad. Carol. Leop. XII. 1825.
43. LANGE, JULIUS, Über Entwickelung der Oelbehälter in den Früchten der Umbelliferen. Diss. Koenigsberg. 1884.
44. LE LANESSAN, J. L., Observations sur le developpement du fruit des Ombellifères. Bull. Soc. Linn. Paris **1**:17–18; 23–24. 1889.
45. LINNÈ, CARL, Genera plantarum. 1737 and 1742.
46. MARTEL, E., Contribuzione all' anatomia del fiore delle Ombellifère. Mem. Reale Accad. Sci. Torino **55**:271–283. 1905; Sur l'anatomie de la fleur des Ombellifères. Jour. Bot. **19**:85–87. 1905.
47. MEYEN, F. J. F., Über die Secretionsorgane der Pflanzen. Königl. Soc. Wiss. Göttingen. p. 21. Berlin. 1837.
48. MEYER, A., Über die Entstehung der Scheidenwände in dem Sekret-führenden plasmofreien Intercellularraume der Vittae der Umbelliferen. Bot. Zeit. **47**:341–352; 357–366; 372–379. 1889.
49. MÖBIUS, M., Untersuchungen über die Morphologie und Anatomie der Monokotylen-ähnlichen Eryngien. Jahrb. Wiss. Bot. **14**:379–425. 1884; **17**:591–621. 1886.
50. MOHL, H. VON, Eine kurze Bemerkung über das Carpophorum des Umbelliferebfrucht. Bot. Zeit. **21**:264–266. 1863.

51. MORISON, ROBERT, Plantarum Umbelliferarum distributio nova. Oxonii. 1672.
52. MOYNIER DE VILLEPOIX, R., Recherches sur les canaux sécréteurs du fruit des Ombellifères. Ann. Sci. Nat. Bot. **5**:348–365. 1878.
53. MÜLLER, CARL, Über phloëmständige Secretkanäle der Umbelliferen und Araliaceen. Ber. Deutsch. Bot. Gessells. **6**:20–32. 1888.
54. NESTEL, A., Beitrage zur Stengel und Blatt Anatomie der Umbelliferen. Diss. Zurich. 1905.
55. NOENEN, F. VAN, Die Anatomie der Umbelliferenachse in ihrer Beziehung zum System. Diss. Erlangen. 1895.
56. PAYER, J. B., Organogenie des familles: Ombellifères. Ann. Sci. Nat. Bot. III. **20**:111. 1853.
57. PETERSEN, E., Undersogelser over Bladnervationes hos Arter af Slaegten *Bupleurum* Tourn. Bot. Tidsskrift **26**:343–376. 1905.
58. REICHARDT, H. W., Über das Centrale Gefässbündel-system einiger Umbelliferen. Sitzber. K. K. Akad. Wissensch. **21**:133–154. 1856.
59. RENNERT, R. J., The phyllodes of *Oxypolis filiformis*, a swamp xerophyte. Bull. Torr. Bot. Club **30**:403–411. 1903.
60. ROSSMANN, G. W. J., Beitrage zur Kenntniss der Spreitenformen in des Familie der Umbelliferen. Abhdl. Naturf. Gesells. Halle. 1864.
61. SCHINDLER, ANT. K., Die Abtrennung der Hippuridaceen von den Halorrhagaceen. Engler's Bot. Jahrb. Beibl. 77. **34**:1–77. 1904.
62. SIELER, T., Beiträge zur Entwicklungsgeschichte des Blütenstandes und der Blüte bei den Umbelliferen. Bot. Zeit. **28**:360–369; 376–382. 1870.
63. SPRENGEL, CURTIUS, Plantarum Umbelliferarum denuo disponendarum Prodromus. N. Schr. Nat. Gesells. II. Halae. 1819; Umbelliferarum genera quaedam melius definita. Berlin Ges. Nat. Freunde Mag. **6**:255–261. 1814.
64. TANFANI, E., Morfologia e istologia del frutto e del seme. Nuovo Giornale Bot. Ital. **23**:451–469. 1891.
65. TITTMANN, J. A., Die keimung der Pflanzen. Dresden. 1821. (pp. 103 seq. and pp. 111 seq., *pls.* 14–16).
66. TERNETZ, C., Morphologie und Anatomie der *Azorella Selago*. Bot. Zeit. **60**:1–20. 1902.
67. TRECUL, AUG., Mémoire sur la formation des feuilles. Ann. Sci. Bot. III. **20**:235 seq. 1853.
68. ———, Des vaisseaux propres dans les Ombellifères. Comptes Rend. **63**:154 and 201. 1866; Des vaisseaux propres dans les Araliacees. Comptes Rend. **64**:886 and 990. 1867.
69. TREVIRANUS, L. C., Symbolarum phytologicarum quibus res herbaria illustratur. **1**:1–92. 1831.
70. ———, Über Fruchtbau und einige Gattungen der Doldengewächse. Bot. Zeit. **19**:9–14. 1861.

71. TREVIRANUS, L. C., and REINHOLD, G., Vermischte Schriften anatomischen und physiologischen inhalts. 4:187. Bremen. 1821.
72. VAN TIEGHEM, PH., Recherches sur la symétrie de structure des plantes vasculaires. Ann. Sci. Nat. Bot. V. 13:5–314. 1870.
73. ———, Sur les canaux oleo-resineux des Ombellifères et des Araliacées. Bull. Soc. Bot. Fr. 19:113–129. 1872.
74. ———, Sur les canaux sécréteurs du pericycle dans la tige et la feuille des Ombellifères et des Araliacées. Bull. Soc. Bot. Fr. 31:29–32. 1884.
75. ———, Mémoire sur les canaux oleo resineux. Ann. Sci. Nat. Bot. 16:96–201. 1872.
76. ———, Sur la germination du *Bupleurum aureum*. Bull. Soc. Bot. Fr. 38:402–404. 1891.
77. ———, Structure de quelques ovules et parti qu'on en peus tirer pour améliorer la classification. Jour. Bot. 12:197–220. 1898.
78. VIGUIER, RENE, Recherches anatomiques sur la classification des Araliacées. Ann. Sci. Nat. Bot. IX. 4:1–207. 1906.
79. VUILLEMIN, P., Sur l'anomalie du système sécréteur des *Hydrocotyle*. Bull. Soc. Bot. Fr. 32:1–14. 1885.
80. WANGERIN, W., Die Umgrenzung und Gliederung der Familie der Cornaceae. Engler's Bot. Jahrb. Beibl. 85. 38:1–88. 1906.
81. WARMING, E., Observations sur la valeur systématique de l'ovule. Mindeskrift Japetus Steenstrup. Copenhagen. 1913.
82. WERNHAM, H. F., Floral evolution with particular reference to the sympetalous dicotyledons. New Phytol. 10:109–120; 145–159; 217–261; 293–305. 1911; 11:145–166; 217–235; 290–305; 373–397. 1912.
83. WETTSTEIN, R., Handbuch der Systematischen Botanik. Leipzig and Wein. 1911.
84. WRETSCHKO, M., Zur Entwickelungsgeschichte des Umbelliferenblattes. Bot. Zeit. 22:305–309; 313–315. 1864.

EXPLANATION OF PLATES XIII, XIV

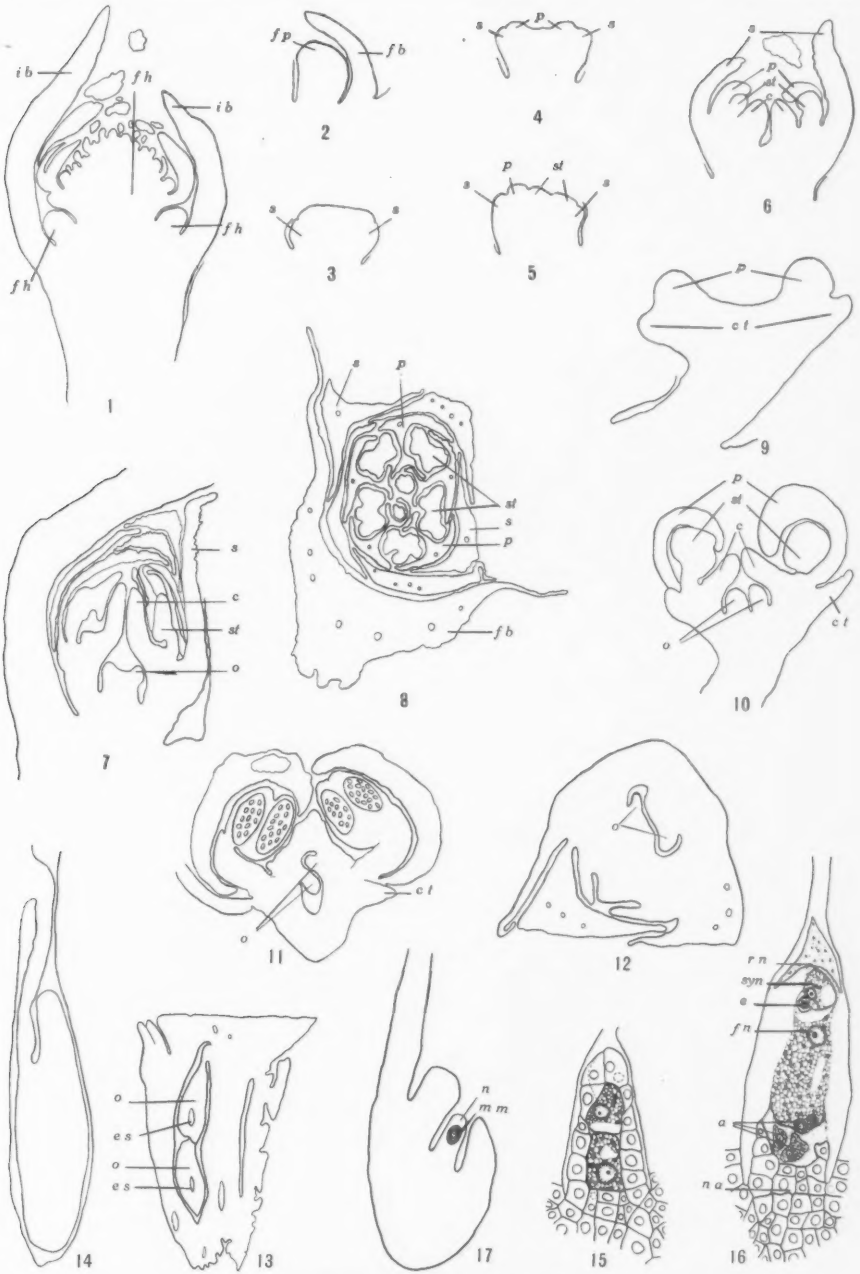
Abbreviations: *fh*, floral head; *ib*, involucral bract; *fb*, floral bract; *fp*, floral papilla; *s*, sepal; *p*, petal; *st*, stamen; *c*, carpel; *ct*, calyx tooth; *o*, ovule; *es*, embryo sac; *mm*, megaspore mother cell; *m*, megaspore; *rn*, remains of nucellus; *na*, nutritive apparatus; *pn*, polar nucleus; *a*, antipodals; *ra*, remains of antipodals; *fn*, fusion nucleus; *e*, egg; *syn*, synergids.

FIG. 1.—A central head of *Eryngium yuccifolium*, showing origin of lateral heads composing umbel.

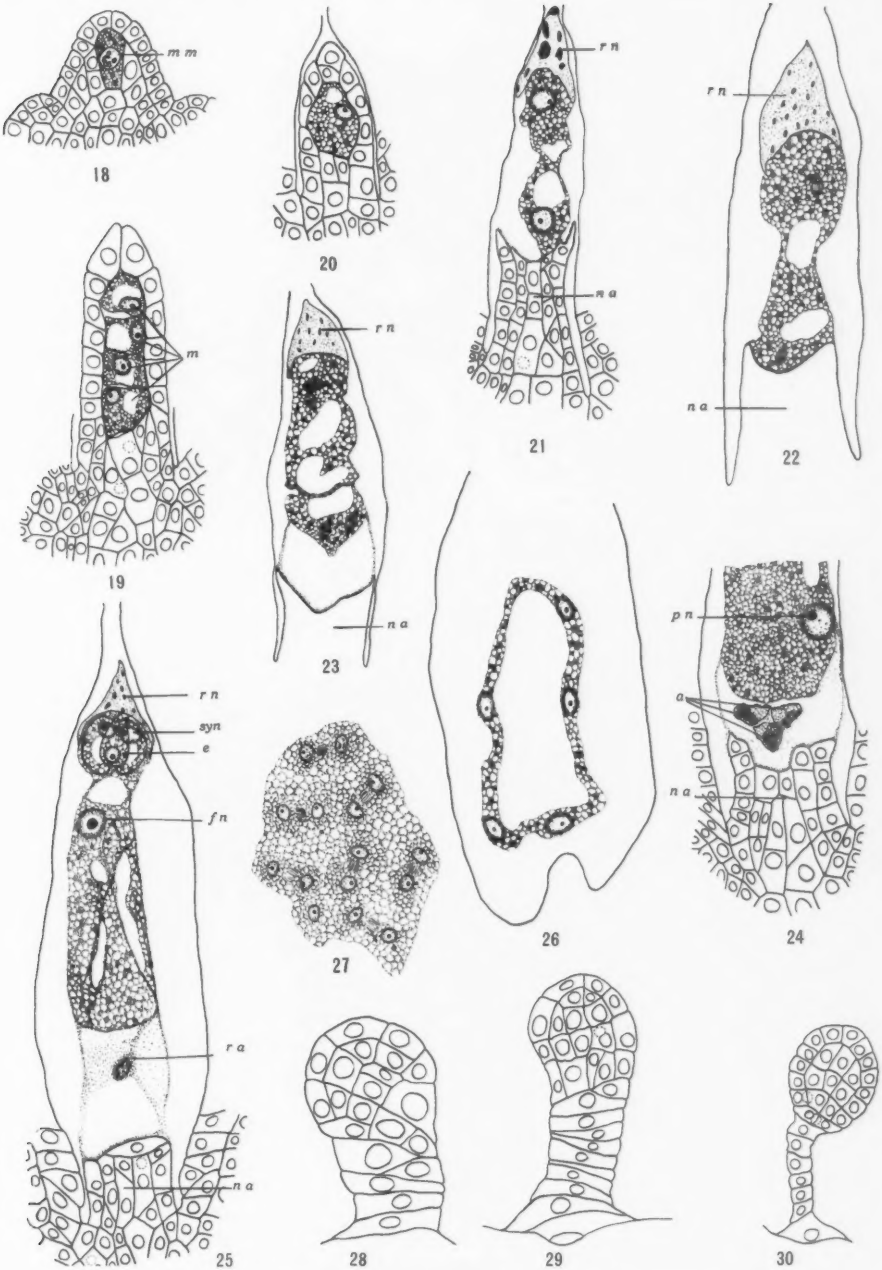
FIGS. 2–7.—Stages in floral development of *E. yuccifolium*.

FIG. 8.—Single mature flower of *E. yuccifolium* in cross-section, showing arrangement of floral cycles.

FIGS. 9, 10.—Stages in floral development of *Zizia aurea*, especially showing calyx teeth.



JURICA on UMBELLIFERAE



JURICA on UMBELLIFERAE

FIG. 11.—Longitudinal section of ovary of *Sium cicutaefolium*, showing the two ovules beginning development in one of ovary cavities.

FIG. 12.—Section of *E. yuccifolium* ovary, showing the two ovules in single ovary cavity; only lower ovule generally develops.

FIG. 13.—Longitudinal section of ovary of *E. yuccifolium*, showing an unusual condition, the developing of two ovules in single ovary cavity.

FIG. 14.—Anatropous ovule of *E. yuccifolium*.

FIG. 15.—Nucellus of *Sium cicutaefolium*, showing two cells, result of first division of megaspore mother cell.

FIG. 16.—Mature embryo sac of *S. cicutaefolium*, showing breaking down of nucellus, egg, one of synergids, fusion nucleus, three antipodals, and nutritive apparatus.

FIGS. 17–30.—*Eryngium yuccifolium*.

FIG. 17.—Ovule showing single integument and nucellus.

FIG. 18.—Nucellus showing megaspore mother cell in synapsis.

FIG. 19.—Nucellus showing four megaspores.

FIG. 20.—Single megaspore from which embryo sac will develop; nucellus beginning to break down.

FIG. 21.—Two-nucleate embryo sac, showing nutritive apparatus.

FIGS. 22, 23.—Two and four-nucleate embryo sac, showing nuclear divisions.

FIG. 24.—Lower end of embryo sac, showing one of polar nuclei, three antipodals, and nutritive apparatus.

FIG. 25.—Mature embryo sac, showing remains of broken down nucellus, egg, synergids, fusion nucleus, remains of antipodals, and nutritive apparatus.

FIG. 26.—Embryo sac in cross-section, showing endosperm formation by free nuclei.

FIG. 27.—Free nuclei of endosperm of embryo sac in longitudinal section.

FIGS. 28–30.—Embryo and suspensor.

DETERMINATION OF MOISTURE CONTENT OF EXPRESSED PLANT TISSUE FLUIDS[1]

ROSS AIKEN GORTNER AND WALTER F. HOFFMAN

The fact that the physico-chemical properties of plant tissue fluids reflect in many instances the ecological environment of the plant, and that the ability of a plant to exist under widely different environmental conditions appears to depend largely upon its ability to adjust the physico-chemical properties of its tissue fluids to the new environment has only recently been recognized. HARRIS (3) has pointed out that any thorough ecological study should include physico-chemical studies of the plant saps. GORTNER and HARRIS (1) have indicated some of the precautions which must be taken in order to secure an accurate measure of the osmotic pressure of expressed plant saps by the cryoscopic method, and in subsequent papers HARRIS and his co-workers (4, 5, 8–14) have investigated the physico-chemical properties of plant saps in a variety of habitats. The physico-chemical determinations which have been used have necessarily been limited to those which are adapted to field laboratory facilities, and which do not require excessive amounts of either time, apparatus, or plant materials. The determinations have therefore been confined exclusively to the measurement of osmotic pressure by the cryoscopic method, the electrical conductivity by the conventional wheatstone bridge, and more recently hydrogen ion concentration (unpublished data).

Throughout all this work it has been recognized that a knowledge of the moisture content of the expressed plant saps would be most desirable. For example, a knowledge of the total solids combined with the depression of the freezing point would permit the calculation of the "average molecular weight" of the dissolved solutes. An increase in "average molecular weight" in a different environment might logically be interpreted as indicating a response

[1] Contribution from the Division of Agricultural Biochemistry, University of Minnesota; published with the approval of the Director as Paper no. 322, Journal Series, Minnesota Agricultural Experiment Station.

to the changed environment by the elaboration of more colloidal materials. In some of the earlier papers (**2, 6, 7**) it was possible to secure these data by the rather laborious procedure of drying weighed portions of the saps in a water oven at 100° and weighing the residue. This method, of course, is wholly unsuited to field studies where hundreds of samples are involved, and is likewise inaccurate, inasmuch as caramelization of the sugars always takes place when plant saps are dried by means of heat. Marked caramelization can only be prevented by drying at room temperature *in vacuo* over sulphuric acid, or by drying in a vacuum oven at not to exceed 50° C. When such methods are employed constant weight is not reached until several days have elapsed.

Another objection to any drying process for field laboratory work lies in the fact that there may be suspended cell débris in expressed plant sap. With abundant laboratory facilities at hand it is comparatively easy to remove such materials by means of a high speed centrifuge or rapid filtration, but in a field laboratory not equipped with a powerful centrifuge, the removal of such débris may be so incomplete as to seriously affect values of dry matter determinations obtained by a drying process.

It recently occurred to one of the writers that it might be possible to determine the moisture content by making use of the refractive index of the plant sap. This method has been employed by sugar manufacturers for many years, and refractometers may be purchased which have a special "sugar scale" from which the percentage of a sugar in a syrup may be read directly.

Tables of refractive indices were consulted and they confirmed this theory, for the refractive indices of solutions of inorganic salts and proteins *in the concentrations normally present in plant saps* appeared to be sufficiently near the values for solutions of carbohydrates so that no excessive errors should result. Accordingly a high grade Abbé refractometer was secured, provided with a special sugar scale, and carefully standardized by the Bureau of Standards, and determinations were made on a series of plant saps with the results shown in table I. This table does not represent selected determinations, but instead every determination which was completed is included, with the exception of two or three where accidents

TABLE I

Leaves of	Refractive index 20° C.	Δ	Moisture by refractometer (percentage)	Moisture over H_2SO_4 *in vacuo*, room temperature (percentage)	Moisture dried 12 hours more at 100° *in vacuo* (percentage)	Moisture dried 6 hours more at 100° *in vacuo** (percentage)	Average molecular weight of solutes
1. Datura Stramonium	1.3433	0.987	92.75	.....	.....	94.18	147
2. Sambucus canadensis	1.3508	1.212	88.00	.....	.....	90.60	210
3. Bryophyllum calycinum	1.3415	0.508	94.10	.....	.....	94.98	230
4. Panicum sp	1.3420	0.984	93.90	.....	.....	94.64	123
5. Zebrina pendula	1.3372	0.370	97.00	98.33	98.42	.....	155
6. Cyperus alternifolia	1.3411	0.956	94.50	95.19	95.55	.....	113
7. Ricinus communis	1.3472	0.747	90.30	90.81	92.39	.....	267
8. Salix sp	1.3571	1.576	84.00	84.34	87.01	.....	227
9. Triticum vulgare var. Marquis	1.3410	0.897	94.50	94.80	95.48	.....	121
10. Triticum vulgare var. Marquis	1.3404	0.857	94.85	94.79	95.51	.....	118
11. Triticum vulgare var. Marquis	1.3409	1.396	94.60	95.09	95.80	.....	77
12. Triticum vulgare var. Marquis	1.3401	0.889	95.00	94.63	95.60	.....	110
13. Triticum vulgare var. Turkey	1.3533	1.276	86.50	86.85	.....	88.94	230
14. Triticum vulgare var. Buffum	1.3561	1.445	84.60	84.67	.....	87.33	236
15. Bryophyllum calycinum	1.3414	0.474	94.10	94.40	.....	95.01	246
16. Cereus sp	1.3400	0.505	95.10	95.27	.....	.....	190
17. Triticum vulgare var. Buffum	1.3600	1.719	82.20	82.85	.....	85.59	234
18. Triticum vulgare var. Minhardi	1.3455	1.147	91.50	91.80	.....	92.62	150
19. Triticum vulgare var. Super	1.3434	1.000	92.90	93.15	.....	93.88	142
20. Triticum vulgare var. Super	1.3474	1.085	90.30	91.53	.....	.....	184
21. Triticum vulgare var. Super	1.3346	.....	98.85	98.86	.....	.....	.....

* Nos. 1, 2, 3, 4 were dried directly in vacuum oven.

happened to the duplicate set being dried either over sulphuric acid or in the vacuum oven.

The tissue fluids were obtained by means of a specially constructed press bowl, and a hydraulic press, after the tissue had been rendered permeable by a preliminary freezing of the tissue for at least eight hours, following the procedure recommended by GORTNER and HARRIS (1) and used in all of the previous work. All saps were centrifuged perfectly clear from suspended débris. We have included in the table values for Δ, the depression of the freezing point (corrected for under cooling), and the "average molecular weight"[1] of the solutes.

Certain of the samples (nos. 9–14 and 17–21) were collected for another purpose by Mr. ROBERT NEWTON, and the significance of the various values will be discussed by him in a later paper. It is sufficient for our purpose to point out the difference between nos. 11 and 14. We have here two wheat saps differing approximately 3 per cent in the freezing point depression (and consequently in osmotic pressure), and at the same time differing by nearly 300 per cent in total solids. This difference can only be due to a difference in colloidal content, a fact that has been proved by NEWTON, using several other methods. Had we been concerned only with determinations of osmotic pressure, electrical conductivity, and hydrogen ion concentration, we might have concluded that these saps possessed practically identical physico-chemical properties, whereas such a conclusion is far from the truth.

Sample no. 21 is a wheat sap dialyzed completely free of sugar and electrolytes, and represents the non-dialyzable colloidal material. It will be noted that the refractometric method measures this colloidal material quantitatively. NEWTON recently had occasion to prepare, in this laboratory, gum acacia sols containing 1, 2, 3, 5, 7, and 10 per cent of highly purified gum acacia, and refractometric readings for total solids on the resulting solutions gave values corresponding with those of the weighed gum acacia which had been added to make the sols.

[1] Calculated by aid of published tables. Cf. HARRIS, J. A., and GORTNER, R. A., Tables of the relative depression of the freezing point, $1860/\Delta$, to facilitate the calculation of molecular weights. Biochem. Bull. 3:259–263. 1914.

One great advantage of the proposed method is that only two or three drops of sap are required for the determination. A film of sap is placed upon the prism, the prism is closed, and as soon as the thermometer inclosed within the prism has reached 20° C. the reading of the moisture content can be made. The entire procedure need not take more than two minutes.

The column "Moisture by refractometer" is read direct from the scale of the instrument, the next column was obtained by weighing 10–20 gm. of the sap into a glass weighing bottle, and drying to constant weight at room temperature in a vacuum desiccator over sulphuric acid. The dried residue varied in color from a clear green to light brown, and still retained the characteristic odor of plant sap.

The next two columns were obtained by heating the residues from the sulphuric acid vacuum treatment in a Freas vacuum oven at 100° under a vacuum of 28 inches for the stated period of time, and again weighing. It will be noted that in each instance there is a higher moisture content indicated by the further drying in the vacuum oven. We believe this to be almost entirely due to the decomposition of carbohydrates, since there was always marked browning or blackening of the residue, and a pronounced burned sugar odor. In no instance was the residue again wholly water soluble, and in every instance the water extracts of the residue were dark opaque brown, with a strong caramel odor. Further proof that this loss of weight is due to decomposition of carbohydrates is afforded by the fact that the loss in weight continues for a long time, and constant weight is in many instances not reached even after seventy-two hours' drying in the vacuum oven at 100° C.

It will likewise be noted that in most instances the refractometer indicates slightly more total solids (that is, less water) than does the drying over sulphuric acid *in vacuo*. In every instance the sulphuric acid in the vacuum desiccator darkened as the drying progressed, indicating that volatile organic compounds were dissolving in the sulphuric acid. It is self evident that esters, alcohols, ethers, and volatile oils are present in all plant saps, and none of these would be estimated by any drying method. We believe, therefore, that at least a part of the excess total solids indicated by the new method are due to such volatile compounds, and that the

refractometer reading more nearly expresses the true value of the moisture content than can be obtained by any known method.

UNIVERSITY FARM
ST. PAUL, MINN.

LITERATURE CITED

1. GORTNER, R. A., and HARRIS, J. A., Notes on the technique of the determination of the depression of the freezing point of vegetable saps. Plant World 17:49–53. 1914.
2. ———, On a possible relationship between the structural peculiarities of normal and teratological fruits of *Passiflora gracilis* and some physico-chemical properties of their expressed juices. Bull. Torr. Bot. Club 40: 27–34. 1913.
3. HARRIS, J. A., Physical chemistry in the service of phytogeography. Science N.S. 46:25–30. 1917.
4. ———, On the osmotic concentration of the tissue fluids of phanerogamic epiphytes. Amer. Jour. Bot. 5:490–506. 1918.
5. ———, On the osmotic concentration of the tissue fluids of desert Loranthaceae. Mem. Torr. Bot. Club 17:307–315. 1918.
6. HARRIS, J. A., and GORTNER, R. A., Physico-chemical properties of vegetable saps. II. A comparison of the physico-chemical constants of the juice of apples and pears of varying size and fertility. Biochem. Bull. 3:196–201. 1914.
7. HARRIS, J. A., GORTNER, R. A., and LAWRENCE, J. V., Physico-chemical properties of vegetable saps. III. A comparison of the physico-chemical constants of the juices expressed from the wall with those from the included capillary whorl in proliferous fruits of *Passiflora gracilis*. Biochem. Bull. 4:52–78. 1915.
8. ———, The relationship between osmotic concentration of leaf sap and the height of leaf insertion in trees. Bull. Torr. Bot. Club 44:267–286. 1917.
9. ———, The osmotic concentration and electrical conductivity of the tissue fluids of ligneous and herbaceous plants. Jour. Phys. Chem. 25:122–146. 1921.
10. HARRIS, J. A., and LAWRENCE, J. V., Cryoscopic determinations on tissue fluids of plants of Jamaican coastal deserts. BOT. GAZ. 64:285–305. 1917.
11. ———, The osmotic concentration of the sap of the leaves of mangrove trees. Biol. Bull. 32:202–211. 1917.
12. ———, The osmotic concentration of tissue fluids of Jamaican montane rain forest vegetation. Amer. Jour. Bot. 4:268–298. 1917.
13. ———, On the osmotic pressure of the tissue fluids of Jamaican Loranthaceae parasitic on various hosts. Amer. Jour. Bot. 3:438–455. 1916.
14. HARRIS, J. A., LAWRENCE, J. V., and GORTNER, R. A., The cryoscopic constants of expressed vegetable saps as related to local environmental conditions in the Arizona deserts. Physiol. Res. 2:1–49. 1916.

MOISTURE CONTENT OF PEACH BUDS IN RELATION TO TEMPERATURE EVALUATIONS

EARL S. JOHNSTON

(WITH TWO FIGURES)

The moisture content of peach fruit buds shows a marked increase in late winter and early spring. Experiments performed at the Maryland Agricultural Experiment Station in 1919 show that moisture values based on the dry weight of buds increased from an average ratio of 0.69 on January 7 to 3.65 on March 28. The following year the average ratio increased from 0.73 on January 29 to 3.51 on April 1. Such marked increases come after the end of the rest period. CHANDLER[1] has shown for several varieties of peach that the rest period ends during the latter part of December or the first part of January. The work of AUCHTER in 1919 (unpublished), at the Maryland Experiment Station, shows that the rest period for fruit buds of six peach varieties was completed by December 25. It is true that there is a fluctuation in the moisture content of dormant buds, but such variations are slight in comparison with those coming at the time the buds start their growth. Concerning buds of the plum, STRAUSBAUGH[2] states:

> During the period of dormancy the moisture content of the semihardy varieties fluctuates with the temperature. Periods of low temperatures are accompanied by a loss of moisture from the leaf and fruit buds, and higher winter temperatures, which are seldom above freezing in Minnesota, by an increase in moisture content.

It is interesting to note that the moisture content of his hardy variety, Assiniboine, remained fairly constant during dormancy.

Since the increase in moisture content following dormancy seems to be related to growth of the bud, an examination has been made of the available moisture data of peach fruit buds and of

[1] CHANDLER, W. H., Winter killing of peach buds as influenced by previous treatment. Mo. Agric. Exp. Sta. Bull. 74. 1907.

[2] STRAUSBAUGH, P. D., Dormancy and hardiness in the plum. BOT. GAZ. 61: 337–357. 1921.

temperature data obtained during the last three years (1919–1921) at the Maryland Station. The 1919 data include those previously reported by JOHNSTON.[3] Fifteen Elberta and fifteen Greensboro trees are averaged for this year's data. The 1920 data have not yet been reported, but are the averages of twenty-seven trees, representing eighteen varieties. The buds of this year were collected from an orchard that had been severely pruned the year previous. The 1921 data are very meager, having been obtained from two trees only, one an Elberta, the other a Greensboro. Moisture determinations, however, were made more frequently during this year.

Temperature evaluations

Three kinds of temperature indices have been applied to these moisture data. In making use of the remainder indices, it is

TABLE I

MOISTURE CONTENT OF PEACH BUDS WITH CORRESPONDING TEMPERATURE INDICES SUMMED FROM JANUARY 1 OF EACH YEAR

DATE	MOISTURE	TEMPERATURE INDICES SUMMED FROM JANUARY 1		
	Ratio of water content to dry weight of buds	Daily mean above 43° F.	Physiological index	Exponential index
1919				
January 7	0.69	14	12.6	2.94
February 7	0.85	22	37.3	16.16
March 7	1.61	55	86.2	31.31
March 28	3.65	173	218.8	59.83
1920				
January 29	0.73	0	2.5	1.00
March 2	0.77	0	10.3	5.16
March 22	1.49	48	66.5	18.86
April 1	3.51	147	177.0	36.12
1921				
January 26	0.86	32	36.4	11.47
February 12	0.99	37	51.0	18.14
February 18	1.13	65	85.0	24.19
February 25	1.16	65	85.3	24.19
March 5	1.46	96	120.7	32.56
March 11	2.27	185	252.3	45.10
March 14	2.72	223	294.0	50.67

assumed that 43° F. (6.1° C.) is the "zero" for growth and other physiological processes, and that daily mean temperatures above

[3] JOHNSTON, EARL S., An index of hardiness in peach buds. Amer. Jour. Bot. 6:373–379. 1919.

43° are "effective" units. These daily mean temperatures (average of daily maximum and minimum) above 43° have been summed from January 1 to each date on which the moisture determinations were made. The physiological summation indices, as derived by LIVINGSTON,[4] were obtained from the daily mean temperatures,

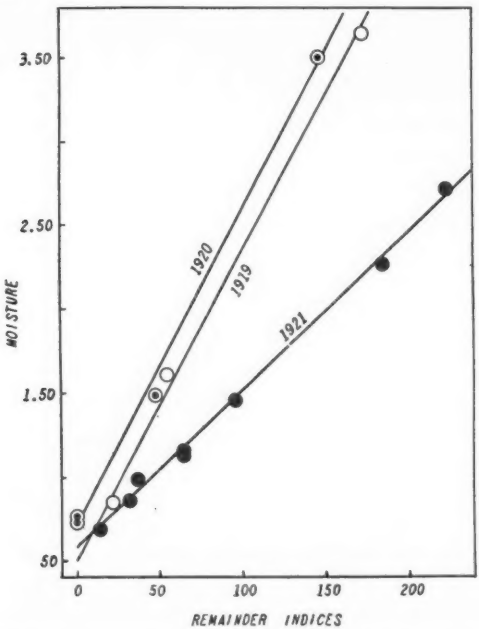


FIG. 1.—Moisture content of peach buds plotted against remainder indices summed from January 1 for years 1919–1921.

and are likewise summed from January 1 to each particular date. The chemical efficiency indices proposed by LIVINGSTON and LIVINGSTON[5] were summed from the indices corresponding to the daily mean temperatures. These three kinds of temperature indices are presented, together with the moisture data, in table I.

[4] LIVINGSTON, B. E., Physiological temperature indices for the study of plant growth in relation to climatic conditions. Physiol. Res. 1:399–420. 1916.

[5] LIVINGSTON, B. E., and LIVINGSTON, G. J., Temperature coefficients in plant geography and climatology. BOT. GAZ. 56:349–375. 1913.

A general survey of the temperature indices in table I at once shows that the 1920 season was colder and less advanced by April 1 than those of 1919 and 1921 on March 28 and March 11, respectively. If the sum of the daily mean temperatures above 43° be used as the criterion, it is seen that the season of 1921 was more advanced on February 18 than the 1919 season on March 7, and the season of 1920 on March 22. The moisture content of the fruit

TABLE II

MOISTURE CONTENT OF PEACH BUDS WITH CORRESPONDING TEMPERATURE INDICES SUMMED FROM JANUARY 1 OF EACH YEAR, EXPRESSED AS NUMBERS RELATIVE TO EACH SEASONAL RANGE, FIRST AND LAST VALUES BEING 0 AND 1.00 RESPECTIVELY

DATE	MOISTURE	TEMPERATURE INDICES SUMMED FROM JANUARY 1		
	Ratio of water content to dry weight of buds	Daily mean above 43°F.	Physiological index	Exponential index
1919				
January 7	0	0	0	0
February 7	0.05	0.05	0.12	0.23
March 7	0.31	0.26	0.36	0.50
March 28	1.00	1.00	1.00	1.00
1920				
January 29	0	0	0	0
March 2	0.01	0	0.04	0.12
March 22	0.27	0.33	0.37	0.51
April 1	1.00	1.00	1.00	1.00
1921				
January 26	0	0	0	0
February 12	0.07	0.03	0.06	0.17
February 18	0.15	0.17	0.19	0.32
February 25	0.16	0.17	0.19	0.32
March 5	0.32	0.34	0.33	0.54
March 11	0.76	0.80	0.84	0.86
March 14	1.00	1.00	1.00	1.00

buds on January 26, 1921, was practically the same as that on February 7, 1919, and somewhat greater than that on March 2, 1920. Such early development of buds increases the danger of fruit loss by spring freezes.

The moisture values given in table I have been plotted against the summation values of the daily mean temperatures above 43° F., and are presented in fig. 1. The slopes of the 1919 and 1920 curves are practically the same, while that of 1921 is quite different. There are apparently some conditioning influences at work before January 1 that determine the slopes of these curves.

After the slopes have once been "predetermined," the increase seems to depend mainly on temperature, either directly or indirectly. It is thus possible to predict the moisture content of the buds for any date after the first of January to the time the pink petals of

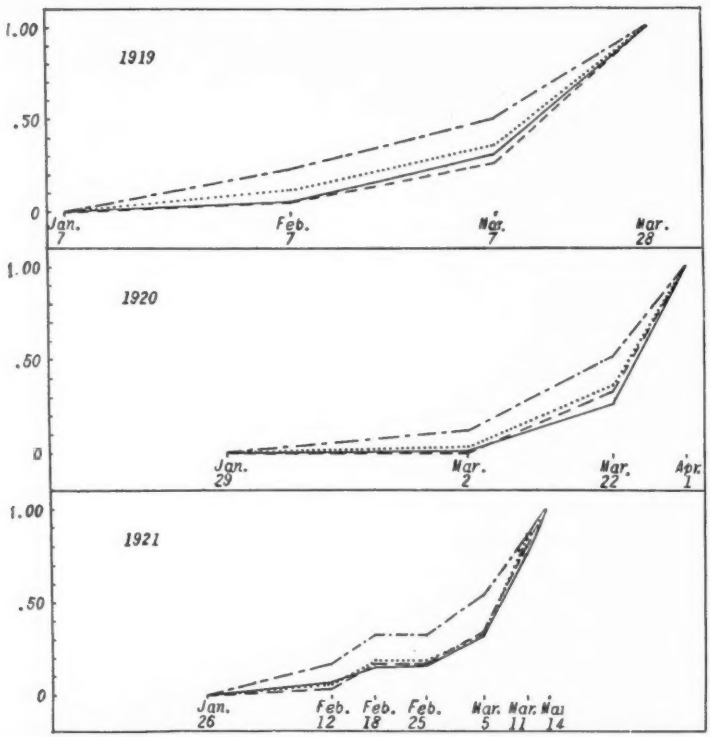


FIG. 2.—Proportionate increase in moisture content of peach buds (continuous lines) and corresponding increases in remainder indices (dash lines), physiological indices (dot lines), and exponential indices (dash-dot lines) for years 1919–1921.

the buds begin to show. The daily mean temperatures and the origin of the slope of the curve must be known. If the origin be considered the first moisture value with its corresponding temperature summation after January 1, or after the end of the rest period, then the slope can be determined from one other later moisture determination.

The other temperature data in table I were also plotted against the corresponding moisture values, but are not presented in graphic form. The curves obtained by using the physiological indices were practically straight lines, and quite similar to those obtained from the daily mean temperatures above 43° F. The relationship between the moisture and the exponential indices, however, was not linear.

The data of table I have been rearranged so that the first reading of each measurement for each year is zero and the last unity. Within this range it is somewhat easier to compare the proportionate amount of change in each measurement. These data are presented in table II, and in the form of graphs in fig. 2. The close agreement between the moisture and the temperature indices representing the summation of daily mean temperatures above 43° F. is readily seen.

Conclusions

It is realized that the observations discussed in this paper are somewhat limited in number, but nevertheless they suggest a definite relationship between air temperature and the rate of increase in the moisture content of peach fruit buds. There can be little doubt, however, that other conditioning influences are operative before January 1, that determine the manner in which these buds responded to temperature.

Agricultural Experiment Station
University of Maryland
College Park, Md.

ALTERNARIA FROM CALIFORNIA

D. G. MILBRAITH

(WITH TWO FIGURES)

Frequent occurrence of a distinct spot on the leaves of cabbage and cauliflower[1] in certain sections of California led to an investigation of its causes and distribution. In culture media, infected leaf tissue taken from the spot yielded constantly a form of *Alternaria* which was found to be a species hitherto undescribed. Macroscopically the infected area appeared smooth, although somewhat sunken, but lacked the profuse growth of conidia and the zonation which characterize the lesions produced on leaves of cabbage by *Alternaria brassicae* (Berk.) Sacc. The disease was found to be restricted to that district of the state lying directly south of San Francisco, where high relative humidity and a uniform temperature prevail. In this district there have been planted annually about 2000 acres of cabbage and cauliflower for shipment to eastern markets, and in view of the frequency of the occurrence of the disease it may be the cause of a pathological problem in transit as well as in the field.

The first symptom of the disease is the appearance of small black specks about 1 mm. in diameter. Often countless specks appear simultaneously in the laminae. Infection occurs almost entirely on apparently vigorous leaves, young as well as old; and under conditions of high relative humidity the initial lesion may develop into a spot ranging from 0.5 to 1 mm. in diameter in fourteen days. The spots are circular, somewhat sunken, black with a purplish cast, the center being darker than the margin (fig. 1). The color depends largely upon the color of the leaf, for upon pale green leaves, as found on Winningstadt and Cannon-ball cabbage, lighter spots are produced than on the dark green varieties of cabbage. Sporulation of the fungus is sparse on still vigorous leaves, but becomes profuse on yellow and detached leaves.

[1] The term cauliflower includes both cauliflower and broccoli.

The causal fungus grew luxuriantly on all common culture media. Sporulation was abundant, and the mycelium was greatly suppressed. *En masse*, the color of the colony is light olivaceous on starchy media and dark olivaceous on nitrogenous media.

FIG. 1.—Cabbage leaf showing abundant spots caused by *Alternaria oleracea;* note absence of zonation characteristic of many *Alternaria* spots.

Zonation in the colony was not visible. A description of the fungus is as follows:

Alternaria oleracea, n. sp.

Hyphae short, greatly subordinated, straight, sparsely branched, occasionally septate, very light olivaceous to hyaline, average 4.5 μ in diameter; conidiophores short, light olivaceous, short

branched, branches almost even in length, about 35 μ; conidia olivaceous to brown, echinulation none in culture four months old, tapering very slightly to apical cell, tapering frequently absent, apical cell frequently hyaline, conidia catenulate, as many as eight in one chain, catenulation both on host and on culture media, longitudinal septation very rare, considerable constriction of the walls at septation, size of the conidia taken from artificially inoculated cabbage leaf 29.7–61.2 μ by 8.7–12.3 μ, average size 43.7 by 10.5 μ. Conidia developed on standard lima bean agar measured on the seventh day as follows: 1-septate, 13.4–16 μ by 6.5–7.5 μ; 2-septate, 17.5–18.7 μ by 8.7 μ; 3-septate, 20–26.5 μ by 8–12 μ; 4-septate, 34–36 μ by 12.5–14 μ; 5-septate, 30–38 μ by 9.5–14 μ; 9-septate, 64.7–70 μ by 10–14 μ (fig. 2).

Inoculation experiments with pure cultures were made on growing cabbage and cauliflower plants in the field and in pots under control in glass cages, at the field station of the Office of Cotton, Truck, and Forage Crop Disease Investigations, located at Alhambra, California. The varieties of cabbage used in these experiments were Winningstadt, Late Drumhead, Early Jersey Wakefield, and Fat Dutch, and the varieties of cauliflower were two selections commonly grown in the San Francisco district. In Alhambra the relative humidity of the atmosphere is considerably lower than in the San Francisco district, where the disease is prevalent. On account of this variation, open field inoculation did not progress above a small speck. When the inoculated leaf was covered with a wax paper sack in which there had been suspended a water-soaked mass of gauze, lesions of normal size were produced readily. Successful infections were produced by spraying conidia, suspended in water, on uninjured leaves, and by wounding and inserting conidia. Infections were obtained more rapidly in glass cages where high relative humidity could be maintained. Within seven days lesions ranging in diameter from 1 cm. to 1.5 cm. were produced. Similar inoculation experiments were made with *Alternaria brassicae* obtained from old cauliflower and cabbage leaves, and with *Alternaria* sp. from spots on tomato fruit. The former produced spots with definite zonations, while the latter failed to grow on cabbage and cauliflower.

Black leaf spot of cabbage has been attributed to the fungus *Alternaria brassicae* for a long time. HARTER and JONES[2] state that the black spot fungus may attack the cabbage plant at any stage of its growth, but it is not common except on the older leaves in the field or on heads in storage. In the field it appears on the lower or outer leaves of the maturing plant as distinct, roundish,

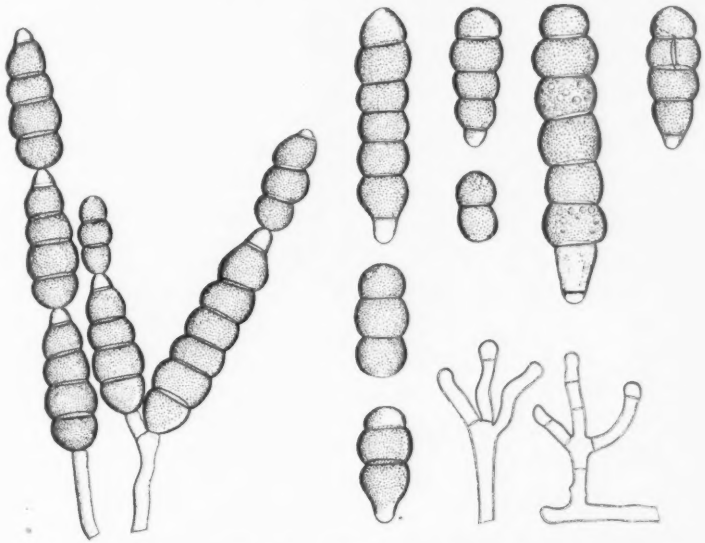


FIG. 2.—Types of conidia and conidiophores of *Alternaria oleracea* from cabbage leaf.

black spots, commonly marked with concentric brown zones. SACCARDO[3] describes the fungus *A. brassicae* (in translation) as follows:

Hyphae short, compact, little branched, even growth, in tufts; conidia deciduous, elongate, fusoid, clavate, size 60–80 μ by 14–18 μ, 6–8 septate, muriform, olivaceous; fungus found on decaying and old leaves of *Brassica oleracea*.

[2] HARTER, L. L., and JONES, L. R., Cabbage diseases. U.S. Dept. Agric. Farmers' Bull. 925. *figs. 13.* 1918.

[3] SACCARDO, P. A., Sylloge Fungorum 4:546. 1886.

MASSEE and COOKE give similar descriptions, except that COOKE employs the term *Macrosporium brassicae* Berk., and describes the conidia as antennaeform, rather longer than the peduncle. ELLIOTT[4] concluded that *A. brassicae* is morphologically similar to *A. solani* (E. and M.) Jones and Grout, and placed both in the same group, which he based upon having long, narrow, regular, tapering spores with few longitudinal septae. It appears that the form of *Alternaria* which the writer dealt with differs from *A. brassicae* both morphologically and in the form of spot produced on cabbage. In view of this difference, it is highly probable that a new species is involved in the production of the leaf spot found in the San Francisco district, and it is suggested that it be named *Alternaria oleracea.*

DEPARTMENT OF AGRICULTURE
WASHINGTON, D.C.

[4] ELLIOTT, JOHN A., Taxonomic characters of the genera *Alternaria* and *Macrosporium.* Amer. Jour. Bot. 4:439–476. *pls. 19–20.* 1917.

BASISPORIUM GALLARUM MOLL., A PARASITE OF THE TOMATO[1]

G. B. RAMSEY

(WITH ELEVEN FIGURES)

While studying the various diseases of the tomato which are found under transit and market conditions, the writer became interested in an unusual fungus which was isolated from the California crop of November 1919. Observations have been made in the Chicago market during the past three seasons to see whether the fungus recurred on California tomatoes, or whether it could be found on those from Florida, Cuba, Mexico, and other tomato shipping districts, but this particular fungus has not been isolated again. The potential seriousness of this fungus as a wound parasite of the tomato, however, and the fact that it has not been reported as a plant pathogen, seemed to make it desirable to publish this note.

The original isolation was made from a soft, red, blister-like lesion near the blossom end of a ripe tomato. A luxuriant growth developed upon the nutrient agar plate, and a great number of spores were formed within a few days. The characteristic smooth, black, subspherical spores borne singly upon their club-shaped sporophores made it comparatively easy to place the fungus in the genus *Basisporium*.

Basisporium gallarum was established as a new genus and species in 1902 by MOLLIARD,[2] in order to properly locate and describe a hyphomycete which he had found upon dead larvae of *Lipara lucens* Meigen, within galls which this insect produces on *Phragmites communis* Trin. He does not mention having found this fungus before, or of later finding it upon any other host. So far as the

[1] Contribution from Research Laboratory on Market Diseases of Vegetables and Fruits; Department of Botany of University of Chicago and United States Department of Agriculture cooperating.

[2] MOLLIARD, M., Bull. Soc. Myc. France, p. 167. 1902.

writer has been able to ascertain, no mention has ever been made in the literature of the parasitism of *Basisporium* upon plants.[1]

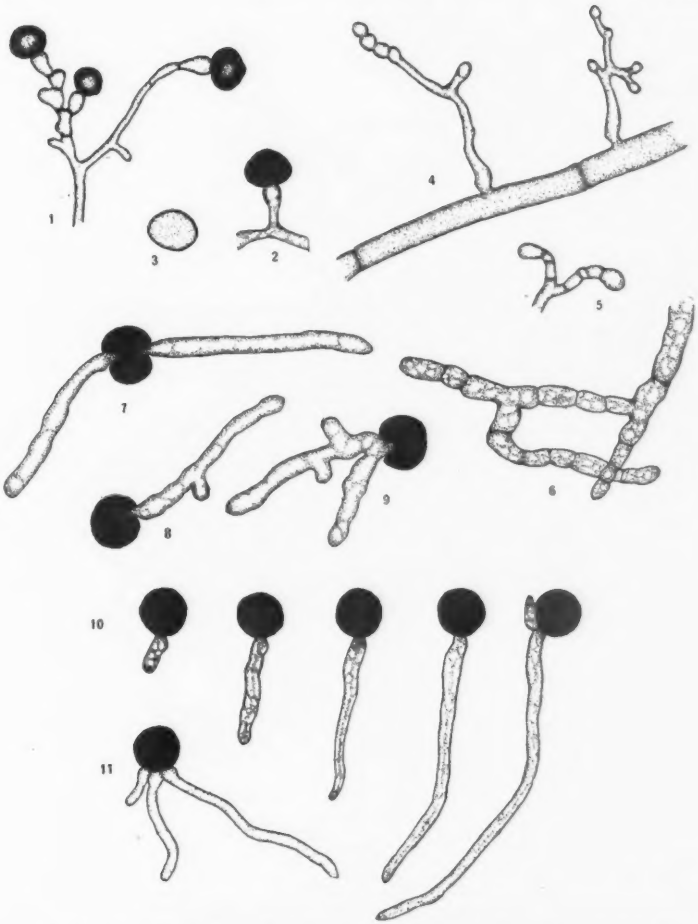
Numerous inoculation experiments have shown conclusively that the fungus is strongly pathogenic to the fruits of the tomato. Pure culture re-isolations of *Basisporium* have been obtained many times from the advancing edge of characteristic lesions produced upon fruits inoculated with a transfer from the original culture. Single spore cultures have been made and used in all experiments described, in order to avoid all possibilities of contaminating organisms.

All attempted inoculations upon unwounded surfaces of both ripe and green tomatoes have failed. Inoculations in wounded surfaces of ripe fruits have always produced the characteristic decay described. Soft, red lesions, two inches in diameter, have been produced on ripe fruits held in a moist chamber at room temperature, within four days. An abundance of pale, smoke colored mycelium is developed in a humid atmosphere. Ripe fruits inoculated in such wounds do not decay at a temperature of 9°–10° C. Specimens have been held two weeks without showing signs of decay when kept at this temperature.

Inoculation experiments with mature green tomatoes have proved positive. It is quite evident, however, that the fungus would grow much more rapidly and produce rot more quickly in ripe fruits. Green tomatoes examined after being inoculated and kept in moist chambers at room temperature for five days showed only slight surface discoloration at the wound. The locule underneath, which was turning pink, showed discoloration and decay to a depth of one-half inch.

The spores of *Basisporium* germinate readily in nutrient solutions. In freshly expressed juice of green tomatoes, as well as that of the ripe fruits, 90–100 per cent germination is obtained within twenty-four hours at 20°–21° C. At 34°–35° C. practically all spores germinate within twenty-four hours; while at 9°–10° C. only about one-half germinate in the green juice, and 80–90 per cent in

[1] In a letter, Miss A. E. JENKINS of the Office of Pathological Collections, Washington, D.C., reports *Basisporium* as having been found on cultures from corn, wheat, and dewberries.



FIGS. 1–11.—Fig. 1, sporophore bearing three slightly immature spores; fig. 2, mature spore; fig. 3, microtome section through mature spore showing thickness of opaque wall; fig. 4, large hypha on surface of agar plate bearing two young fertile hyphae; fig. 5, showing method of branching and spore formation at tips of fertile aerial hyphae; fig. 6, large sterile mycelium taken from pulp of infected tomato; figs. 7–9, spores germinated at room temperature in freshly expressed juice of ripe tomatoes; fig. 10, showing hourly growth of germinating spores in Standard Nutrient Salt Solution, at room temperature; fig. 11, spore germinated in sterile distilled water at room temperature. (All drawings made with the aid of a camera lucida.)

the ripe juice. It is interesting to note in this connection that although the spores germinate readily in fresh tomato juice at 9°–10° C., decay is practically if not totally prevented in both ripe and green tomatoes at this temperature.

In sterile distilled water at 10° C. only a very few spores have ever been observed to germinate. After three days, usually less than 1 per cent have germinated. At room temperature about 10 per cent germinate within twenty-four hours, and 20–30 per cent in forty-eight hours. The spores seem to absorb water rapidly, and burst before having a chance to send forth a germ tube when placed in sterile distilled water at 34°–35° C.

Basisporium grows well on most nutrient agars. Potato agar plus 2 per cent dextrose has been used with excellent results in culture studies of this fungus. The mycelium is white at first, moderately thick and cottony, later turning pale smoke color as sporulation takes place. Fine growth has been obtained at a temperature as high as 35° C., but sporulation seems to be inhibited somewhat at that temperature. Plate cultures held at 9°–10° C. for seven days developed only a very thin, flat growth whose radius was 3 mm. Taking the other temperature experiments into consideration, it would seem that 10° C. is approximately the minimum at which *Basisporium* will grow.

Spores are borne abundantly on the terminal as well as the lateral branches of the small fertile hyphae. The spores are black, subspherical, smooth, and have a tendency to be slightly conical on top and flattened underneath. Average measurement through the axis is 11.4 μ, and through the transverse diameter 15.3 μ. The fertile aerial hyphae are 3.5–5 μ in diameter. Large sterile hyphae within decaying tissues of tomatoes and on surface of agar in plate cultures are 12–17 μ in diameter. These measurements show a slight variation from those given by MOLLIARD. The original drawings also show a narrower constriction of the sporophore at its junction with the spore. These differences are felt to be of minor importance, however, and, although a new host is involved, do not seem to justify the making of a new species.

BUREAU OF PLANT INDUSTRY
WASHINGTON, D.C.

BRIEFER ARTICLES

SAGENOPTERIS, A MESOZOIC REPRESENTATIVE OF THE HYDROPTERACEAE

(WITH ONE FIGURE)

The question of the botanical relationship of the genus *Sagenopteris* has been a debated one for a great many years. The genus was founded by PRESL in 1838 for a Rhaetic (upper Triassic) form from south Germany, and since that time a considerable number of species have been described from all over the world, all of which come from rocks of Mesozoic age. The genus has been discussed by SCHIMPER, NATHORST, BERRY, and SEWARD. Nearly all students have recognized its pteridophytic affinities, and many have suggested a relationship with the so-called water ferns (Hydropteraceae). NATHORST was the most emphatic advocate of the latter relationship, which was based upon habit and venation of the vegetative parts. He fortified it by his discovery in the Rhaetic beds near Palsjö in Sweden of fruitlike bodies, which, aside from their resemblance to the sporocarps of the modern *Marsilea*, did not represent the fruits of any known Coniferophyte or Cycadophyte present in the Rhaetic flora of Sweden, and therefore were considered to be the sporocarps of *Sagenopteris*, being found in association with the fronds of *S. undulata* Nathorst.

Somewhat similar remains, considered to represent the sporocarps of *Sagenopteris*, have been recorded by ZIGNO from the Jurassic of Italy, and by HEER from the Upper Cretaceous of western Greenland (HEER referred the latter to *Marsilea*). In describing the Lower Cretaceous of Maryland in 1911, I considered the evidence sufficiently good to warrant referring the Potomac species of *Sagenopteris* to the Hydropteraceae. The most abundant of these Potomac species, and the only one at all well characterized, namely *S. elliptica* Fontaine, has been found to have a considerable geographic range. It is not only present in the Patuxent formation (the oldest member of the Potomac group), but occurs also in the Patapsco formation, which there is good evidence to consider to be of Albian age. This species has also been recorded from the Knoxville and Horsetown beds of California, the Kootenay formation of Montana, and the Lower Cretaceous of Queen Charlotte Islands.

The occasion for the present note is the presence of this species (*S. elliptica* Fontaine) in the lower part of the Blairmore formation of western Canada, in association with characteristic sporocarps which are almost identical with those described by NATHORST from much older beds on the other side of the world. The supposed sporocarps from the Blairmore formation (fig. 1) probably belong to the same botanical species as *S. elliptica*, but in view of the fact that this has not been demonstrated, and in accordance with paleobotanical usage, they may be named **Sagenopteris canadensis,** sp. nov. It may be remarked parenthetically that not all of the recognized species of *Sagenopteris* based upon foliar remains are clear cut, the European Jurassic *S. Phillipsii* being not obviously different from *S. rhoifolia*, or from the European Lower Cretaceous *S. Mantelli*. The last is scarcely, if at all, to be distinguished from, and has often been confused with, the American *S. elliptica*.

Fig. 1.—Sporocarps of *Sagenopteris canadensis;* ×4.

The sporocarp, which it is believed belongs to the latter, may be described as follows: Sporocarp hard and resistant, stalked, bean-shaped, gibbous, slightly flattened at the sides, more recurved and slightly more narrowly rounded at one end, about 5 mm. in length, and about 3 mm. in height, with fifteen or sixteen transverse encircling veins which are impressed, and appear as sulcae in the material, retaining more carbonaceous matter because thicker, and appearing blacker than the remainder of the wall; bands between these impressed veins lighter in color, and with a thin central line more or less developed.

SEWARD in his latest work refers *Sagenopteris* to the Hydropteraceae tentatively, summing up his remarks with the statement that "decisive evidence as to its position in the plant kingdom is at present lacking; the inclusion of the genus as a possible member of the Hydropterideae has still to be justified."

It would seem that the discovery of bodies that have all the megascopic features of sporocarps, that cannot be referred to any other known elements of the associated flora, in association with foliage, which in habit, form, and venation independently suggests comparisons with the genus *Marsilea*, at two such remote localities as Sweden and western Canada, is strong presumptive proof of relationship. Moreover, these two occurrences are very different in age, thus showing no obvious change in the sporocarps during the time that elapsed between the Rhaetic and the mid-Cretaceous, a time interval of at least several million years, and comparable in magnitude with the time that has elapsed from the mid-Cretaceous to the present. If these sporocarps preserve their appearance during the older interval, this conservative feature becomes an argument of validity in comparing their latest occurrence with the *Marsilea* sporocarps of the present.

The evidence, then, that *Sagenopteris* is related to the recent Hydropteraceae is about as conclusive as we can hope to secure in the absence of structural material, which is present in about 0.0001 per cent of the cases with which the paleobotanist has to deal.—EDWARD W. BERRY, *Johns Hopkins University, Baltimore, Md.*

A BISPORANGIATE SPOROPHYLL OF LYCOPODIUM LUCIDULUM

(WITH ONE FIGURE)

The occurrence of more than a single sporangium on a sporophyll in *Lycopodium* is so unusual that it is believed the following account will be of interest.

BOWER[1] records a case in which a sporophyll of *L. rigidum*, from a specimen in the Glasgow University Herbarium, bears "two sporangia of slightly unequal size placed side by side. They are individually smaller than the average sporangia in the near neighborhood on the same axis." BOWER's statement would hold equally true for a similar case in *L. lucidulum* recently found in the writer's laboratory. As will be noted from fig. 1, the larger of the sporangia shows the normal kidney shape typical of the sporangium of *Lycopodium*, while the smaller has more the form of a football. The relative thicknesses of the two stalks correspond closely to the size of the sporangia. Both stalks are slightly lateral to the

[1] BOWER, F. O., Note on abnormal plurality of sporangia in *Lycopodium rigidum* Gmel. Ann. Botany 17:278–280. *figs. 18.* 1903.

midrib of the leaf, the smaller being the farther removed. Normally the stalk of the single sporangium is immediately above the midrib of the leaf.

The origin of such a situation can only be conjectured. Since the sporangium of *Lycopodium* arises from a transverse row of initials, it is probable in this case that there was a sterilization of archesporial tissue within this row, separating the two ends of the row, each of which developed to maturity in a practically normal manner, producing the two distinct and separate sporangia.

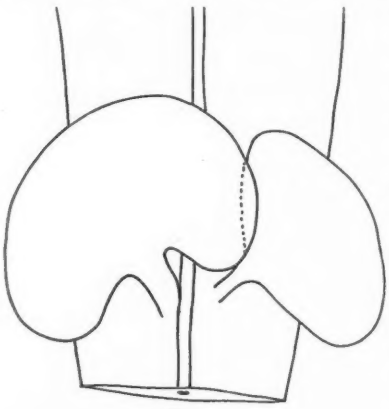


FIG. 1

The formation of the "subarchesporial pad" in the sporangium of *Lycopodium* may be regarded as a sterilization of potentially sporogenous tissue, which, while not producing complete septation of the sporangium, is suggestive as a prelude to the complete septation which occurs in other forms, such as *Psilotum*, as a result of the sterilization of complete plates of tissue. In this case the sterilization may be thought of as having occurred so early as to result in two completely distinct sporangia. Whether the polysporangiate condition in Pteridophytes may have arisen in a similar way is of theoretic interest, the synangium being regarded as intermediary between the monosporangiate and the polysporangiate conditions. On the other hand, the two sporangia may have arisen from two distinct groups of initials.

BOWER, concluding his note on *L. rigidum*, states that "it shows how even the most rigid facts of morphological experience are liable to exception, and that this applies equally to spore-bearing members, in cases where their forms seem most stereotyped."

To summarize, (1) bisporangiate sporophylls in *Lycopodium* are very rare, a single case of each being known in *L. rigidum* and *L. lucidulum;* (2) the two sporangia may have arisen as a result of very early sterilization of archesporial tissue, or from two distinct groups of initials.—A. W. DUPLER, *Juniata College, Huntingdon, Pa.*

A SIMPLE APPARATUS FOR CONTROLLING TEMPERATURES

(WITH ONE FIGURE)

In developing the control equipment for a humidity chamber in which it was desired to control temperatures over long periods of time, it was found essential to use a temperature control apparatus activated by an electric current taken from the ordinary lighting circuit. Troublesome failures with dry cells and storage batteries led to the construction

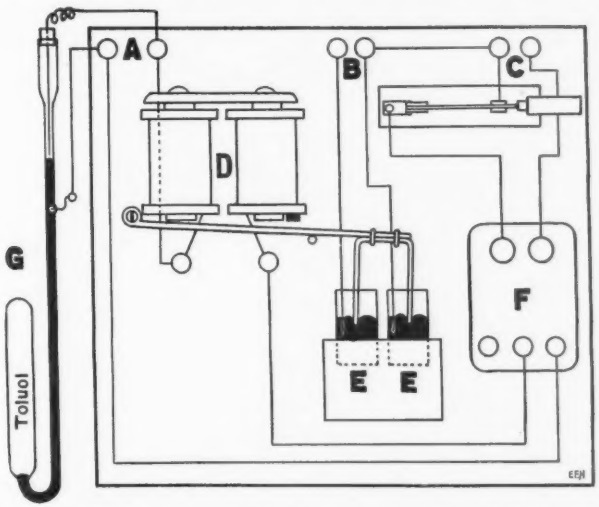


FIG. 1.—*A*, binding posts to which wires from thermostat tube (*G*) are attached; to left-hand binding post (*B*) is attached one of the wires leading from heating unit; *C*, binding posts to which wires from main lighting circuit are attached (one of the wires leading from heating unit is attached to right-hand post); *D*, "transformer bell" electro-magnetic coils wound for alternating current with bell and clapper removed (to extreme right of armature bar is attached a piece of U-shaped heavy copper wire); attached to lower part of right-hand magnet is a small piece of rubber cut from a rubber stopper, which projects far enough beyond magnet bar to eliminate most of the "chatter" or "buzz" when coils are operating; *E*, two glass vials (0.75×1.5 inches) partly filled with mercury, into which points of the U-shaped copper wire are immersed; wires leading from *B* are in contact with the mercury in vials; *F*, small, 3 volt, bell-ringing, alternating current transformer which furnishes 6, 8, or 14 volts as desired (here connected to furnish 8 volts, alternating current); *H*, small single-blade knife switch used to test action of coils at *D*; the asbestos-covered board upon which units *A* to *F* are mounted measures 9.5×11.5 inches, and is designed for wall attachment; use of transite board or other insulating material and mounting to reduce fire risks is recommended.

of the apparatus shown in fig. 1. This is a modification of a somewhat similar instrument secured through Dr. K. F. KELLERMAN, and used for some time at the Laboratory of Forest Pathology at Madison, Wisconsin, in controlling the temperature of a Bausch and Lomb incubator.

The main advantage of the apparatus here described lies in the fact that the same current (110 volts, 60 cycle, alternating) which passes through the heating units is used, after reducing the voltage to 6, 8, or 14 volts, to operate the relay (fig. 1*D*). The apparatus is simple, comparatively cheap, and when once set up and adjusted needs little attention. It will stand continuous service for long periods of time, and when used in connection with a toluol and mercury filled tube (fig. 1*G*) controls temperatures within a range of a quarter of a degree Fahrenheit.

When the mercury column in the tube (*G*) rises and contacts with the fine wire held by the cork, the coils at *D* draw the armature bar up to the poles of the magnet, the U-shaped wire is pulled free from the mercury at *E*, and the current supplying the heating unit is broken, thus shutting off the heat. A reversal of this action turns the heat on again.

At *E* the make and break of the current supplying the heat units is very positive in action. There is no arcing of the current between the mercury and the wire points, so long as the U-shaped wire is raised sufficiently above the mercury surface. The small voltage of the current passing through the mercury column in the tube (*G*) reduces the spark to a minimum, and for this reason little trouble is encountered with arcing, vaporizing of mercury, or clouding of the tube where contact is made between the fine wire and the mercury. A metal cap with adjusting screw carrying a fine platinum wire may be substituted for the cork and wire as shown.—ERNEST E. HUBERT, *Laboratory of Forest Pathology, Bureau of Plant Industry, in cooperation with the Forest Products Laboratory, Madison, Wisconsin.*

CURRENT LITERATURE

BOOK REVIEWS

Yeasts

A few years ago a group of French savants arranged for a new *Encyclopédie Scientifique*. Under the direction of TOULOUSE, some of the most distinguished scientists are attempting to prepare a series of complete and readable monographs covering the whole range of science. The plan calls for about a thousand volumes, classified under forty main sections of knowledge.

If GUILLIERMOND'S[1] volume on yeasts is a fair sample, this encyclopedia will be indispensable to every school of science. This volume includes an ample bibliography, index, and analytic table of contents. The introduction defines the yeasts, morphologically and physiologically, and places them as "a family of the Ascomycetes, known by the name of Saccharomycetes." A brief historical résumé concludes that three names will always be connected with the study of yeasts, PASTEUR, HANSEN, and BUCHNER.

The first chapter deals with the morphology of vegetation and reproduction. The second one, on cytology, discusses the nucleus, metachromatic bodies, and other particles, both in vegetation and reproduction. The metachromatic bodies are stated to be composed probably of a compound of nucleic acid. They are identical with A. MEYER'S volutin grains. Two chapters on physiology are rich in material. The subjects of the following chapters are phylogeny, methods of study and culture, identification of species, variation of species, and classification. The second part is a systematic description of the known genera and species. Constant reference throughout all the chapters to the little known Schizomyces and Zygosaccharomyces adds much to the interest of the work. Every page of this admirable volume is full of clear, terse statements of observation, and carefully balanced discussion. It is rare for as keen an investigator to have such a lucid and logical style.

The translation of GUILLIERMOND by TANNER[2] is really a new edition. In bringing the work up to date, GUILLIERMOND has been responsible for the morphology, phylogeny, and classification, and TANNER for the physiology. Most of the text, however, is carried over without change. Unfortunately, the translation has been poorly done. Almost every page of the English

[1] GUILLIERMOND, A. T., Les Levures. II. pp. 565. Paris: Octave Doin et fils. 1912.

[2] GUILLIERMOND, A. T., The yeasts. Translated and thoroughly revised in collaboration with the original author by F. W. TANNER. pp. xix+424. New York: John Wiley and Sons. 1920.

edition furnishes distressing mistranslations. When the French idiom *ne . . . . que* is translated as a flat negative, the author is made to say the exact reverse of what he means, and the rest of the paragraph becomes absurd. The French use of the article has been carried over literally, even where it fits the English idiom! Again, where the author says, for example, a fluid *may reach* a certain concentration, the faithful translator puts it, *is able to reach*. "Matières hydrocarbonnes" becomes "hydrocarbon materials," in heavy-faced type; and the paragraph discusses sugar, cellulose, and glycogen! Perhaps the consistent reference to WAGER as WAGNER in the text is an error of proofreading, for the name is correct in the bibliography and in the French text. The American book is very well printed, with large, open, legible type, but no one can safely use it without having a copy of the original at hand, or at least without having enough knowledge of French to read the original author's meaning between the lines.—H. S. CONARD.

NOTES FOR STUDENTS

Electrons in photosynthesis.—An attempt has been made by DIXON and POOLE[3] to interpret photosynthesis in terms of the electronic theory. On the basis of photo-electric phenomena in sensitizers of photographic films, and the absorption spectra of chlorophyll, they believe that the first action of light is to disturb electrons in the chlorophyll molecule. Experiments were made to determine whether the electrons were actually ejected by the incident radiation, or whether the disturbances were too weak to do more than displace the electrons within groups of atoms, or from molecule to molecule of chlorophyll.

By delicate electrometer measurements they were able to establish the occurrence of a slight photo-electric effect in chlorophyll under illumination, but this effect is apparently produced by ultra violet radiations, not by those of visible frequencies and synthetic activity, for they find that the effect is magnified about 2000 times by use of a light rich in ultra violet rays. Quantitative use of the data showed that possibly 75 electrons per square centimeter per second might be ejected from a layer of chlorophyll by light from a 500 watt lamp. In terms of energy, this effect is utterly negligible, for the actual synthesis of food in plants goes on at a rate which would require about nine trillion times as much energy as these ejected electrons could supply. It is necessary to conclude, therefore, that ejection of electrons is not significant in photosynthesis, and that the chlorophyll cannot ionize substances external to itself by electronic bombardment. If this be true, then the synthetic reactions must concern the chlorophyll molecule itself, and the electrons are merely shifted from atom to atom, or molecule to molecule, as in ordinary chemical reactions. These shiftings, of course, will alter linkages, and change the chemical character of atomic groups, probably rendering inactive groups of atoms reactive. Such

[3] DIXON, HENRY H., and POOLE, HORACE H., Photosynthesis and the electronic theory. Sci. Proc. Roy. Dublin Soc. **16**:63–77. 1920.

a conception favors the chemical theories of photosynthesis which assume that chlorophyll itself enters into the reactions, rather than those which assume that the synthetic reactions are performed externally to the chlorophyll by means of energy absorbed and transformed by the pigments.

That the light actually displaces electrons seems to be proved by DIXON and BALL[4], who show that the chlorophyll acts as a sensitizer of photographic films at the temperature of liquid air, a temperature believed to be too low for chemical reactions other than electronic displacement. They suggest that chlorophyll *a* and *β* might have an important connection in the synthetic process, as indicated by the following equations:

$$\underset{\text{(chlorophyll } a\text{)}}{C_{55}H_{72}O_5N_4Mg} + CO \rightarrow \underset{\text{(chlorophyll } \beta\text{)}}{C_{55}H_{70}O_6N_4Mg} + HCHO$$

$$\underset{\text{(chlorophyll } \beta\text{)}}{C_{55}H_{70}O_6N_4Mg} + H_2O \rightarrow \underset{\text{(chlorophyll } a\text{)}}{C_{55}H_{72}O_5N_4Mg} + O_2$$

The fact that *in vitro* experiments with chlorophyll *a* and CO_2 do not yield formaldehyde could be explained by accepting WILLSTÄTTER'S assumption that before the CO_2 will react with the chlorophyll it must first be combined into a carbamino acid, which can then be decomposed by the reactive group in the chlorophyll, which group was rendered reactive by the electronic shifting due to light.

Regardless of whether the discovered facts are sufficient to establish the relations between electronic displacements and synthesis of carbohydrates, the attempt made by the authors to carry over into physiological interpretations the newer conceptions of electron chemistry is praiseworthy, and will be followed with great interest by physiologists. Ultimately all the chemical processes of life must be interpreted along similar lines.—C. A. SHULL.

The mycoplasm theory.—In two brief notes in English and an extended discussion in German, ERIKSSON[5] makes a spirited defense of his mycoplasm theory. Only incidental reference is made in these papers to the work on the grain rust, on which the theory was established, but the previously published conclusions on the downy mildew of spinach, the late blight of potato, and the hollyhock rust are reaffirmed, critics are replied to, not without acerbity, and in the case of the hollyhock rust new observations and experiments are adduced which, the author believes, still further support his hypothesis, of which he is not only the originator, but has been, to date, almost the sole protagonist. In

---

[4] DIXON, HENRY H., and BALL, NIGEL G., Photosynthesis and the electronic theory. II. Notes Bot. School, Trinity Coll., Dublin **3**:199–205. 1922.

[5] ERIKSSON, JAKOB, The mycoplasm theory, is it dispensable or not? Phytopath. **11**:385–388. 1921.

———, The life of *Puccinia malvacearum* Mont. within the host plant and on its surface. Phytopath. **11**:459–463. 1921.

———, Das Leben des Malvenrostpilzes (*Puccinia malvacearum* Mont.) in und auf der Nährpflanze. Handl. Kungl. Svensk. Vetensk.-Akad. **62**[5]:1–190. *figs. 31*. 1921.

the first paper he lists additional pathogens which he believes have a mycoplasm stage in their life histories, making a total of fifteen rusts, four downy mildews, three powdery mildews, one slime mould, and the causal agent of tobacco mosaic for which this curious combination of host and fungous protoplasm is claimed as proved or suspected.

Additional data are presented which lead the author to reaffirm, with some modifications, his previously expressed belief in the physiological dimorphism of the teleutospores, as evidenced by their mode of germination; one sort, in moist air, giving rise to long hyphae terminating in chains of conidia; the other kind, under the same conditions, germinating in ordinary teleutospore fashion, producing basidiospores. The latter, on penetrating the host tissue, give rise at once to an intercellular mycelium from which new sori develop in a few days, and are therefore the agencies by means of which the rapid spread from plant to plant is effected. On the other hand, the conidia discharge their contents into a host cell, with the contents of which they form an intimate intracellular protoplasmic union, that is to say, a mycoplasm, which multiplies within the host, even entering the embryo, thus forming the hibernating stage of the fungus. In spring, with the renewal of growth in a dormant plant or the germination of a seed, the fungus element separates out from the mycoplasm, organizes an intercellular mycelium, and eventually produces sori. Numerous observations are reported tending to show that the rapid spread of the disease from plant to plant occurs only late in the year, when basidiospores are being produced, the summer (conidial) infections not appearing until the following season. A series of experiments is reported in which the host plants were watered with weak solutions of copper sulphate. This resulted in a perceptible diminution in the number of summer pustules which appeared, these being due, according to the view outlined, to the conidial infections of the previous year, the fungus wintering over within the host. The copper solution was of no avail against the late summer basidiospore infection. Endeavors to inject the copper solution into the leaves and stems were unsuccessful. Without attempting to summarize the voluminous data, it may be admitted that the evidence seems to favor the view that the fungus may winter over within the host tissues. This is by no means a proof, however, that it is in a state of mycoplasmic symbiosis with the host. Furthermore, it must be regarded as a serious oversight that no notice whatever is taken of the carefully planned experiments of BAILEY,[6] which seem to point very decidedly to conclusions quite opposed to those of ERIKSSON. Certainly the facts in the case must be explained, and the mycoplasm theory admittedly offers a theoretical explanation. The widespread opposition to it is based on the feeling that the facts may ultimately be accounted for satisfactorily without the theory. If this shall prove to be impossible, the mycoplasm theory offers itself as a working hypothesis, which, however, must be confirmed by far less ambiguous cytological evidence than has yet been presented before it can be regarded as established fact.—G. W. MARTIN.

[6] Ann. Botany **34**: 173–200. 1920.

White pine blister rust.—The present state of our knowledge concerning this most important tree rust is ably summarized by SPAULDING[7] in a recent contribution from the Bureau of Plant Industry. The extensive scope of the treatment is partially indicated by the bibliography of 180 titles. The opinion is expressed that *Cronartium ribicola* is of Asiatic origin, that it spread throughout Europe during the nineteenth century, and was introduced into North America on young trees of *Pinus Strobus* after 1900. The life history of the fungus and its relations with its various hosts are exhaustively discussed. It is regarded as established that the overwintering is chiefly by the mycelium in the bark of living pines, and that it is in this stage that the long migrations have taken place, but it is also recognized that the fungus may overwinter on *Ribes*. The attempts which have been made to control the disease are reviewed, and the conclusion is reached that its eradication is impossible except in the case of small isolated advance infections, but that the systematic removal of all *Ribes* in white pine forest areas will keep the disease in check, and that this method is both practicable and profitable.

ERIKSSON[8] renews his contention that the Peridermiums on *Pinus Strobus*, *P. silvestris*, and *P. Cembra* are biological races of a single species in which the specialization is not yet definitely fixed. He believes that he has evidence that the white pine blister rust is transmitted by diseased seed and also from pine to pine. The direct evidence presented is not of the sort that can be regarded as final. To supplement it, so far as the matter of the spread from pine to pine is concerned, great emphasis is laid upon the undoubted autoecism of closely related species, with particular stress upon the results of HAACK. These, it is interesting to note, are characterized as worthless by SPAULDING. The latter admits, however, that the work of MEINECKE and of HEDGCOCK on *Peridermium cerebrum*, and that of KLEBAHN on *Peridermium pini* "throw doubt on the strict heteroecism of the aeciospores of all stem-inhabiting pine Peridermiums." This is very far from proving the autoecism of the aecidiospores of *Peridermium strobi*, as ERIKSSON seems to assume. The point is obviously one of fundamental importance, and it is to be hoped that the experiments now under way in this country will shortly throw more light upon it. ERIKSSON finds in recent American and European experiences of the overwintering of the fungus on *Ribes* a complete justification of his own early expressed belief concerning this phase of the problem. That such overwintering occurs cannot longer be doubted. That it is of any great importance in the perpetuation of the disease is still very questionable.—G. W. MARTIN.

Isoetes.—OSBORN[9] has obtained some very interesting results from an investigation of *Isoetes Drummondii*, a species widely distributed in certain

[7] SPAULDING, PERLEY, Investigations of the white pine blister rust. Bull. 957. U.S. Dept. Agric. pp. 100. *pls. 6. figs. 13.* 1922.

[8] ERIKSSON, JAKOB, The connection between *Peridermium strobi* Kleb. and *Cronartium ribicola* Dietr., is it obligate or not? A critical review. Arkiv Botanik 18[2]: 1–40. *figs. 6.* 1922.

[9] OSBORN, T. G. B., Some observations on *Isoetes Drummondii* A. Br. Ann. Botany 36: 41–54. *figs. 15.* 1922.

parts of South Australia. "It grows terrestrially in seasonal swamps during the period of winter rainfall. During the dry summer it aestivates, as do the other geophytes with which it is associated." The stock is buried, and during the vegetative season only a small rosette of linear leaves is visible above the soil. On approach of the dry season, the leaves dry up and become detached, leaving their tough bases and sporangia upon the stock, wholly buried and invisible.

This species seems to be unique among Pteridophytes in its method of spore liberation. There is a special mechanism for freeing the spores which depends for its action upon saturation with water, not upon dryness (as in other Pteridophytes). Other peculiar features of the species are in the nature of preparation for this remarkable method of spore dispersal. In his summary, OSBORN describes the performance as follows: "Early in the rainy season, the hardened bases of the sporophylls are forced above the surface of the soil in a projectile-like mass, carrying with them the sporangia, by the expansion of certain pads of mucilage cells formed at the close of the previous vegetative season on the extreme bases of the sporophylls and from the superficial cells of the leaf-bearing cortex. About the same time the leaves of the new vegetative season begin to appear. The imbricate mass of sporophyll bases breaks up upon the surface of the soil, and the spores are set free by a tearing away of the sporangium wall from its attachment to the sporophyll when sodden. This is due to a difference between the tension of the inner and outer surfaces of the sporangium wall when saturated, and results in an eversion of the wall."

TAKAMINE[10] has investigated the gametophytes of *Isoetes japonica* and *I. asiatica*, with some interesting results. The female gametophyte of *I. japonica* usually has five or six archegonia, but sometimes ten or more. When fertilization occurs in one of them, the others degenerate; but in rare cases when fertilization occurs in two or more archegonia, several embryos are developed up to certain stages. Occasionally megaspores and microspores were found in the same sporangium. In *I. asiatica* the $2x$ chromosome number is twenty-two, while in *I. japonica* it is "forty-three to forty-five." Hybrids of the two species were produced, an account of which is promised later.—J. M. C.

Complexmutation.—As the term mutation is now being used by geneticists, its application is restricted to "locus changes" on the chromosomes. At one place on one chromosome, mutation takes place, the effect of the change being so restricted as to involve only a single factor; other factors, although lying very close on the same chromosome, remain unchanged. Save for "deficiency," noted by BRIDGES[11] (which is evidently of a different category), all mutation seems to have been of this very localized type. It is perhaps surprising that no clear cases of mutations involving simultaneous changes in

[10] TAKAMINE, N., Some observations in the life history of *Isoetes*. Bot. Mag. Tokyo **35**: 184–190. *figs. 9.* 1921.

[11] BRIDGES, C. B., Deficiency. Genetics **2**: 445–465. 1917.

several factors in one region of a chromosome have been discovered. NILSSON-EHLE[12] now claims to have such a case, and calls it "complexmutation." Normal wheat mutates to bearded speltoid, involving a simultaneous change in two closely linked factors. Among the F_2 progeny of normal×mutant appear a few bearded normal type and beardless speltoid, but only a very few, due to the very close linkage of the two mutated factors. In another case the investigator claims that three linked factors have mutated simultaneously.—M. C. COULTER.

Ozark forests.—The Ozark region, as covered by PALMER[13] in this reconnoissance, is defined as occupying the southern half of Missouri, a narrow spur crossing southern Illinois, the northwestern part of Arkansas, and a long triangular strip in eastern Oklahoma. The two topographic divisions of this uplifted region, lying midway between the higher mountains of the east and west, are the flat-topped dome of the northern plateau with an average altitude of 300–500 m., and the southern Boston Mountains with a few points above 600 m. It is a hill region surrounded by fertile plains, and possessing a rather abundant rainfall. Floristically there are no distinct floras corresponding to the topographic divisions, although the southern parts of the region, including the Boston Mountains, have a heavier forest growth richer in types than the northern, and include such southern forms as *Aesculus discolor*, *Tilia floridana*, *Rhamnus caroliniana*, *Ilex decidua*, and *Magnolia acuminata*.

The larger portion of the report is occupied by floristic notes on various sections of the flora and on certain genera and species. The author is convinced that in the region as a whole there is a demonstration of the gradual but actual encroachment of forest upon prairie lands.—GEO. D. FULLER.

Temperature and nodule development.—Using soil temperatures ranging from 12° to as high as 40° C., JONES and TISDALE[14] have studied the effect of these temperatures on the development of nodules by alfalfa, red clover, soy beans, and field peas. The results as to the number of nodules developed were not so very consistent, but when the dry weight of the nodules was determined, it was found that the greatest development, in the case of the soy bean, was at 24° C. This effect of temperature on nodule development is not correlated with a corresponding effect on root and shoot development. It is pointed out in the paper that the real question in a study of this kind is not the effect of temperature on the number of nodules developed by the plants, or on the volume of these nodules, but the effect on the amount of nitrogen fixed in the nodules.

---

[12] NILSSON-EHLE, H., Multiple Allelomorphe und Komplexmutationen beim Weizen. Hereditas 1:277–311. 1920.

[13] PALMER, E. J., The forest flora of the Ozark region. Jour. Arnold Arboretum 2:216–232. 1921.

[14] JONES, F. R., and TISDALE, W. B., Effect of soil temperature upon the development of nodules on the roots of certain legumes. Jour. Agric. Res. 22:17–31. *pls. 1–3.* 1921.

Some data in regard to this point are presented. While this is a preliminary paper, the results obtained and the statements as to methods of attack on a problem of this kind are suggestive to workers in this field.—S. V. EATON.

Response of apple trees to nitrogen fertilizers.—Nitrogenous fertilizers have been found by many investigators to cause increased vegetative growth of fruit trees and increased yields of fruit, but the experiments establishing these facts have been largely empirical. A bulletin by HOOKER[15] records the results of experiments planned to get at the chemical changes in the tree resulting from nitrogenous fertilizers. He finds that the spring application of nitrogenous fertilizers causes increased setting of apples, accompanied by an increased nitrogen content of the spurs. In the case of non-bearing trees, increased vegetative growth is caused. Different kinds of quickly available fertilizers had much the same effect. At the time of fruit bud differentiation, the spurs of the spring fertilized trees showed less starch than the spurs of the check trees, so that the spring application of nitrogenous fertilizers would not be expected to favor this process. The accumulation of nitrogen in the spurs just before growth starts in the spring is the greater the later the nitrogenous fertilizers have been applied the preceding season. It is a pleasure to see the horticulturists thus attacking the fundamental problems of their subject.—S. V. EATON.

Vegetation of Illinois.—The recent publication of the 14th annual volume of the Transactions of the State Academy of Science[16] shows about one-third of the volume devoted to reports of botanical and plant ecological studies. The State Forester, R. B. MILLER, discusses some of the undeveloped forest resource, of the southern portion of the state, and in collaboration with GEO. D. FULLER examines in some detail the conditions of tree growth and the forest types existing in a portion of Alexander County. W. G. WATERMAN makes a preliminary report on the bogs of the northern portion of the state, while W. B. MCDOUGALL contributes an interesting key to some forest tree roots. A more extensive investigation is reported by H. DEFOREST, who has studied the woodlands along the Rock River in the northwestern portion of the state. MARY E. RENICH contributes a study of growth as related to size of seed, and A. B. REAGAN has some interesting notes on the plants of the Bois Fort Indian Reservation, Minnesota.—GEO. D. FULLER.

Mosaic disease of tobacco.—PALM[17] has investigated the mosaic disease of tobacco, and has reached the conclusion that it is due to a causal organism.

---

[15] HOOKER, H. D., Certain responses of apple trees to nitrogen applications of different kinds and at different seasons. Mo. Agric. Exp. Sta. Res. Bull. 50. 1–18. 1922.

[16] Transactions of the Illinois State Academy of Science. Fourteenth annual meeting. 1921. **14**: pp. 326. 1922.

[17] PALM, B. T., Is the mosaic disease of tobacco a chlamydozoonose? English translation by P. G. WILSON. Bull. Deliproefstation Medan-Sumatra. no. 15. pp. 10. 1922.

His preparations of the diseased tissue show fairly large irregularly shaped corpuscles and very small granules, both clearly foreign elements, being entirely absent in healthy tissue. The author agrees with IWANOWSKI as to the probable interpretation of the foreign elements, namely, "that the minute granules are very small bacteria, carriers of the virus, and further that the irregularly shaped corpuscles must be considered as a pathological product of reaction of the virus carrier on the cell plasm." This causal organism is thought to be a species of *Strongyloplasma*, and is named *S. Iwanowski* in honor of its original discoverer. —J. M. C.

Effect of age on plant structure.—Miss TELLEFSEN[18] has studied the effect of age upon certain tissues of *Salix nigra*. This species was chosen chiefly because of the comparative ease with which specialized tissues, as roots, will develop from the meristematic tissue of cuttings under laboratory conditions. Numerous tables of measurements are given, and some of the conclusions are as follows. Cuttings from younger trees rooted in less time than those from older trees, and also leaves appeared earlier. Epidermal and cortical cells of the root become smaller as the parent tree becomes older, and xylem and meristematic become larger. The average area of vein islets in leaves from older trees is smaller than average vein islet areas of leaves from younger trees, the amount of vascular tissue increasing with senility, thus reducing the average area of vein islets.—J. M. C.

Leaf-skin theory.—Miss SAUNDERS[19] has reached the conclusion that the surface tissue of the seed plant shoot is of foliar origin, meaning that the leaves are decurrent, not merely those that are usually called so, but all leaves. In the same way the superficial tissue of the hypocotyl are derived from the cotyledons. This so-called leaf-skin is formed by the "downward growth and extension of the leaf primordium, which keeps pace with the extension of the central axis with which it is fused. In the case of flowering stems the leaf-skin is formed by the bracts (when present) and the outermost sepals." Miss SAUNDERS has gone into many details as to the extension of a single leaf surface in relation to the different types of phyllotaxy, the various surface patterns developed, and other features.—J. M. C.

Intrafascicular cambium in monocotyledons.—Mrs. ARBER,[20] in continuing her investigations of the occurrence of intrafascicular cambium in monocotyledons, has added Alismaceae, Aponogetonaceae, and Hydrocharitaceae to the list of monocotyledonous families, in some member of which this tissue has been

---

[18] TELLEFSEN, MARJORIE A., The relation of age to size in certain root cells and vein islets of the leaves of *Salix nigra* Marsh. Amer. Jour. Bot. **9**: 121–139. 1922.

[19] SAUNDERS, EDITH R., The leaf-skin theory of the stem: a consideration of certain anatomico-physiological relations in the Spermatophyte shoot. Ann. Botany **36**: 135–165. *figs. 34.* 1922.

[20] ARBER, AGNES, Studies on intrafascicular cambium in monocotyledons. V. Ann. Botany **36**: 251–256. *figs. 8.* 1922.

observed, bringing the number of such families to twenty-two. Incidentally, she shows that LIGNIER was probably mistaken in attributing only phloem-forming activity to the cambium in the petiolar bundle of *Arum maculatum*, since she finds secondary xylem in the corresponding bundles of a closely related species (*A. italicum*), and LIGNIER'S figures indicate that the same process takes place in *A. maculatum*.—J. M. C.

Heat of inversion.—A careful measurement of the heat of inversion of sucrose by invertase has been made by DIXON and BALL,[21] who used a thermocouple differential calorimeter in vacuum flasks for the determination. Their results confirm the value found by BROWN and PICKERING many years ago, the mean of all results being 3.83 calories for each gram molecule of sugar inverted. The method is more accurate than the heat of combustion determinations of this value.—C. A. SHULL.

Fungus in Pellia.—RIDLER[22] has described the life history of a fungus occurring in a definite zone in the thallus of *Pellia epiphylla*. It was found to occur in the cells of the sporophyte, from which it was isolated, and identified as a species of *Phoma*. The fungus kills the protoplasts of the infected cells of the gametophyte, which ultimately become brown. The effect on the sporophyte is twofold: the contents of the cells are killed, and the cell walls are also wholly or partially absorbed.—J. M. C.

Mycorhiza of conifers.—MCDOUGALL[23] has identified two mycorhizal fungi from the roots of *Picea rubra* as belonging to the genus *Cortinarius*, and described a tubercle-like mycorhiza of *Pinus Strobus*. He reiterates his opinion that these ectotrophic mycorhizal fungi are of no benefit to the trees concerned, and probably do them no great harm, although truly parasitic in their relationship.—G. D. FULLER.

Rocky Mountain flora.—RYDBERG[24] has continued his studies of the montane regions of the southern Rockies, already noted in this journal,[25] by investigating the aquatic and grassland associations, as well as the flora of the sand hills, dry ridges, and rock slides. The plants of these habitats are listed as eastern, western, and endemic.—GEO. D. FULLER.

---

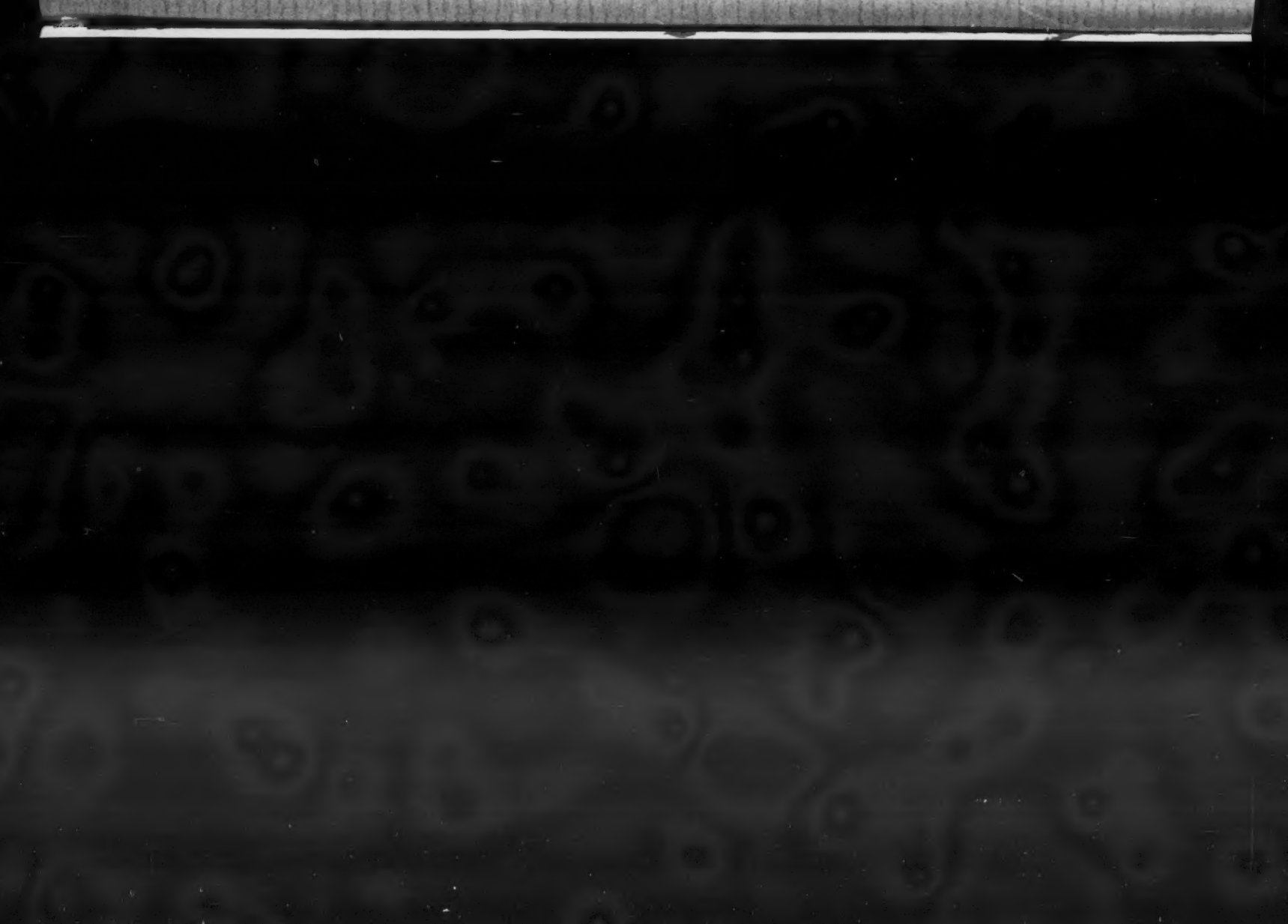
[21] DIXON, H. H., and BALL, NIGEL G., A determination by means of a differential calorimeter of the heat produced during the inversion of sucrose. Notes Bot. School, Trinity Coll., Dublin **3**:121–132. 1922.

[22] RIDLER, W. F. F., The fungus present in *Pellia epiphylla* (L.) Corda. Ann. Botany **36**:193–207. *figs. 8*. 1922.

[23] MCDOUGALL, W. B., Mycorhizas of coniferous trees. Jour. Forestry **20**:255–260. *figs. 3*. 1922.

[24] RYDBERG, P. A., Phytogeographical notes on the Rocky Mountain region. X. Grasslands and other open formations of the montane zone of the southern Rockies. Bull. Torr. Bot. Club **48**:315–327. 1921.

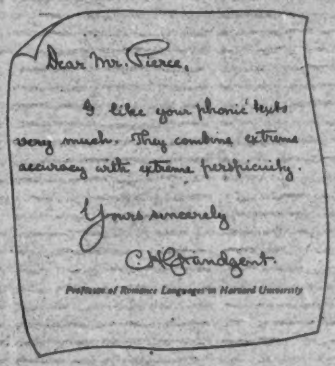
[25] BOT. GAZ. **71**:336. 1921.



WORLD-ROMIC SYSTEM

MASTERKEY TO ALL LANGUAGES

PRIMERS, edited or selected by ROBERT MORRIS PIERCE, 16 languages, $1.94 postpaid, each language: Arabic, Chinese, Danish, Dutch, English, Scotch English, French, German, Italian, Japanese, Panjabi, Polish, Portuguese, Russian, Spanish, Swedish.


Dear Mr. Pierce,
I like your phonic texts very much. They combine extreme accuracy with extreme perspicuity.
Yours sincerely
C. H. Grandgent.
Professor of Romance Languages in Harvard University

CHARTS, by ROBERT MORRIS PIERCE: four, each measuring (paper-size) 24 by 15 inches, and entitled respectively: *Chart of the Universal Alphagam, Chart of the Organs of Speech, Chart of the French Alphagam, Chart of the Spanish Alphagam:* folded and bound in paper $6\frac{7}{8}$ by $4\frac{1}{4}$ inches, 37 cents each, postpaid; mounted on cloth and rollers, for use as wall-charts, $3.88 each, postpaid.

DICTIONARIES, with pronunciations in World Romic, edited or selected by ROBERT MORRIS PIERCE: (*unilingual*) English, French, or German; (*bilingual*) French-English and English-French: $4.86 each, postpaid.

PRONUNCIATION-TABLES, special alphagams, showing, in World Romic, the elementary sounds of 89 languages, edited by ROBERT MORRIS PIERCE, 30 cents postpaid, each language: Afghan, Albanian, Old Armenian, Bearnese (Arrens), Bearnese (Garlin), Bohemian, Bragancian, Brahui, Burgundian, Cantonese, Catalan, Charentese, Cumbrian, Dauphinese, Dubian (Bournois), Dubian (Pontarlier), Dutch, Emilian (Badi), Emilian (Bologna), Emilian (Novellara), Tuscan Emilian (Carrara), Tuscan Emilian (Gombitelli), Tuscan Emilian (Sassalbo), English, American English, British English, Scotch English, Middle English (Orm), Middle English (southern dialect, 12th century), Middle English (East Midland, time of Chaucer), Old English, Finnish, French, West Frisian, Galician, German, Modern Greek, Aeginian Modern Greek, Cycladic Modern Greek, Lesbic Modern Greek (Ajasso), Lesbic Modern Greek (Mitilini), Lesbic Modern Greek (Plomari), Macedonian Modern Greek, Thracian Modern Greek, Old Greek, Hungarian, Old Icelandic, Italian, Japanese, Kanara, Kanauri, Kui, Kurdish, Lahuli, Languedoc, Latin, Laz, Ligurian, Lombard, Lorrainese, Marchesian, Mingrelian, Molisian, Norman, Norwegian, Pahari, Picard, Portuguese, Provençal, Old Provençal, Rhetian, Rumanian, Sanskrit, Scotch, Spanish, Andalusian Spanish, Bosnian Judeo-Spanish, Bulgarian Judeo-Spanish, Mexican Spanish, Tridentan, Umbrian (Gubbio), Umbrian (Rieti), Venetian (Grado), Venetian (Murano), Venetian (Triest), Walloon (Liége), Walloon (Stavelot), Welsh, Yorkshire.

LANGUAGES PUBLISHING COMPANY

Publishers of PIERCE'S LIBRARY: A MISCELLANY OF LITERATURE AND WORKS OF REFERENCE IN ALL LANGUAGES

8 West 40th Street, New York City

REMINGTON PORTABLE

¶ The most *complete* portable typewriter. Has complete *standard keyboard* and other "big machine" features.

¶ Small, light, simple and convenient—fits in case only four inches high.

¶ These surpassing features have made it the leading portable typewriter—in sales and popularity.

Price, complete with case, $60

Remington Typewriter Company
374 Broadway New York
Branches and Dealers Everywhere

A Living Monument to

AGASSIZ

A Joy and Benefit to Students and Lovers of Nature

Please co-operate in the great work of

THE AGASSIZ ASSOCIATION, Inc.

Forty-seven years of effective service
Science, Education and Humanity

The Guide to Nature

Monthly Illustrated Magazine

EDWARD F. BIGELOW, Editor

Subscription, $1.50 per year; single copy, 15 cents; three months' trial, 25 cents

Please write for particulars

ARCADIA
SOUND BEACH CONNECTICUT

SPENCER Mon-Objective Binocular Microscopes

ARE EQUIPPED WITH

I. Interchangeable Tubes—Binocular and Monocular.

II. Nosepiece Adapter mounted independently of the tubes, so that the objectives remain in place and in center irrespective of which tube is used.

SPENCER MON-OBJECTIVE BINOCULAR MICROSCOPE NO. 2H

SPENCER LENS COMPANY

MANUFACTURERS

SPENCER BUFFALO U.S.A.

Microscopes, Microtomes, Delineascopes, Optical Glass, Telescope Discs and Lenses, Optical Measuring Instruments, etc.

BUFFALO, N.Y.

SPENCER BUFFALO U.S.A.